A Thesis in General Surgery

A STUDY OF EXPRESSION OF HER2/neu IN CARCINOMA BREAST WITH REFERENCE TO CLINICOPATHOLOGICAL FEATURES AND PROGNOSTIC INDEX

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APRIL – 2016

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled " **A STUDY OF EXPRESSION OF HER2/neu IN CARCINOMA BREAST WITH REFERENCE TO CLINICOPATHOLOGICAL FEATURES AND PROGNOSTIC INDEX "** is a bonafide and genuine research work carried out by me under the guidance of Dr.S.Balakrishnan, M.S., Professor, Department of General Surgery, Kilpauk Medical College, Chennai.

This dissertation is submitted to THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI in partial fulfillment of the requirements for the degree of M.S. General Surgery examination to be held in April 2016.

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INSTITUTIONAL ETHICAL COMMITTEE
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Protocol ID No.10/11/2015 Dt. 04. 02.2015

CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study of expression HER2/neu in Carcinoma Breast with reference to clinicopathological- features and prognostic index". -For Project Work-submitted by Dr.J.R.Jeena Josephin, M.S.Post Graduate, Department of General Surgery, Govt Kilpauk Medical College, Chennai-10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

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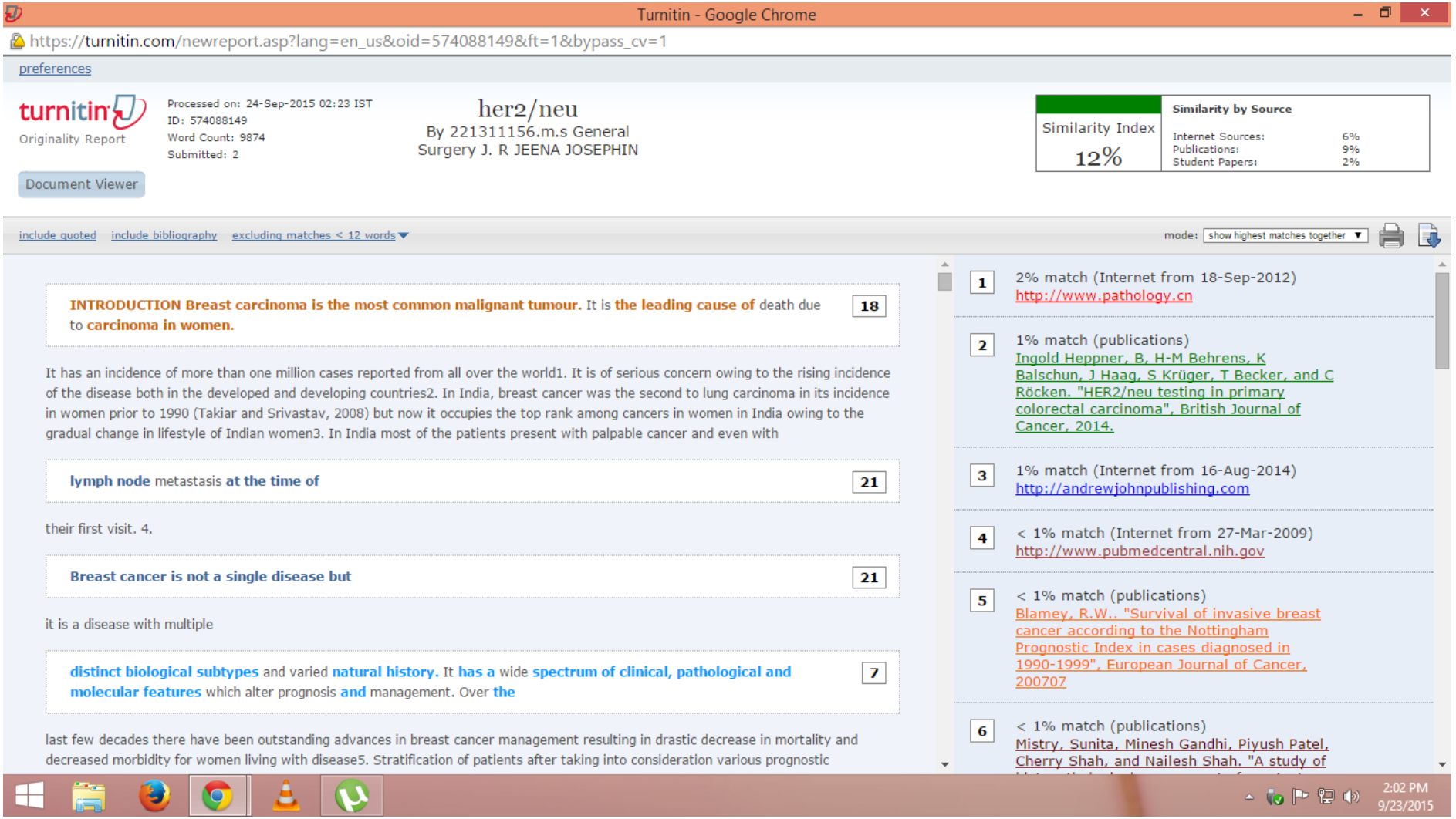
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INTRODUCTION Breast carcinoma is the most common malignant tumour. It is the leading cause of death due to carcinoma in women. 18
It has an incidence of more than one million cases reported from all over the world1. It is of serious concern owing to the rising incidence of the disease both in the developed and developing countries2. In India, breast cancer was the second to lung carcinoma in its incidence in women prior to 1990 (Takiar and Srivastav, 2008) but now it occupies the top rank among cancers in women in India owing to the gradual change in lifestyle of Indian women3. In India most of the patients present with palpable cancer and even with
lymph node metastasis at the time of 21
their first visit. 4.
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it is a disease with multiple
distinct biological subtypes and varied natural history. It has a wide spectrum of clinical, pathological and molecular features which alter prognosis and management. Over the 7
last few decades there have been outstanding advances in breast cancer management resulting in drastic decrease in mortality and decreased morbidity for women living with disease5. Stratification of patients after taking into consideration various prognostic
1 2% match (Internet from 18-Sep-2012) http://www.pathology.cn
2 1% match (publications) Ingold Heppner, B, H-M Behrens, K Balschun, J Haag, S Krüger, T Becker, and C Röcken. "HER2/neu testing in primary colorectal carcinoma", British Journal of Cancer, 2014.
3 1% match (Internet from 16-Aug-2014) http://andrewjohnpublishing.com
4 < 1% match (Internet from 27-Mar-2009) http://www.pubmedcentral.nih.gov
5 < 1% match (publications) Blamey, R.W.. "Survival of invasive breast cancer according to the Nottingham Prognostic Index in cases diagnosed in 1990-1999", European Journal of Cancer, 200707
6 < 1% match (publications) Mistry, Sunita, Minesh Gandhi, Piyush Patel, Cherry Shah, and Nailesh Shah. "A study of
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LIST OF ABBREVATIONS USED

AR	Antigen Retrieval
ASCO- CAP	American Society of Clinical Oncology- College of American Pathologists
CISH	Chromogenic in- situ hybridization
DSM	Deep Surgical Margin
ER	Estrogen Receptor
EPG	Excellent Prognostic Group
FISH	Fluorescent In-Situ Hybridization
Fibro	Fibrosis
GPG	Good Prognostic Group
Her2/neu	Human epidermal growth factor receptor2/neuroblastoma
H & E	Haematoxylin & Eosin
HPF	High Power Field
IDC	Infiltrating Duct Carcinoma
IHC	Immunohistochemistry
ILC	Infiltrating Lobular Carcinoma
LVI	Lymphovascular Invasion
MPG I	Moderate Prognostic Group I
MPG II	Moderate Prognostic Group II
Necro	Necrosis
No.	Number
NOS	Not Otherwise Specified

NPI	Nottingham Prognostic Index
NSBR	Nottingham modification of Scarff Bloom Richardson Grading
PR	Progesterone Receptor
PPG	Poor Prognostic Group
Sl No.	Serial Number
SR	Stromal Reaction
TDLU	Terminal Duct Lobular Unit
VEGF	Vascular Endothelial Growth Factor
VPG	Very Poor Prognosis

ABSTRACT

Background

Carcinoma of the breast has clinical heterogeneity with high morbidity and mortality. Determination of prognostic factors is important in improving the overall survival breast cancer patients. Her2/neu is one of the powerful predictors of poor prognosis. Thus a study of Her2/neu expression estimated by immunohistochemistry in breast cancer is the need of the hour. This study investigates correlations between the presence of Her2/neu and various clinicopathological prognostic parameters in primary breast carcinomas.

Objectives

1. Study of the HER2/neu status in breast carcinomas in the study sample.
2. To find the association of HER2/neu status with clinicopathological prognostic parameters in breast carcinomas in the study sample.
3. To find the association of HER2/neu status with lymph node status.

Methodology

Patients diagnosed as carcinoma breast, admitted in Department of General surgery, Govt. Royapettah Hospital and post operative modified radical mastectomy specimens were examined for gross and microscopic features. Immunohistochemistry was used to study the Her2/ neu expression. Her2/neu status was correlated with various clinicopathological prognostic parameters.

Results

There was a significant statistical association between Her2/neu expression with lymph node status(p=0.012) and presenting symptoms such as pain and nipple discharge. Expression of Her2/ neu expression was increased in tumour size of more than 2 cms (64%) and higher tumour grade (90%). The major histological subtype was Infiltrating duct carcinoma, NOS type. Meanwhile there was no significant association of Her2/neu expression with menopausal status, NPI groups and other histological parameters.

Conclusion

Our study data indicates that Her2/neu may be a more powerful predictor for poor prognosis as its expression is associated with important prognostic parameters like increased tumour size, high tumour grade, high NPI score and lymph node involvement.

Key words

Immunohistochemistry; Her2/ neu; breast carcinoma; clinicopathologicalprognostic parameters; Nottingham modification of Scarff Bloom Richardson grading (NSBR grading); Nottingham Prognostic Index.

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Introduction

INTRODUCTION

Breast carcinoma is the most common malignant tumour. It is the leading cause of death due to carcinoma in women. It has an incidence of more than one million cases reported from all over the world[1]. It is of serious concern owing to the rising incidence of the disease both in the developed and developing countries[2].

In India, breast cancer was the second to lung carcinoma in its incidence in women prior to 1990 (Takiar and Srivastav, 2008) but now it occupies the top rank among cancers in women in India owing to the gradual change in lifestyle of Indian women[3]. In India most of the patients present with palpable cancer and even with lymph node metastasis at the time of their first visit.[4].

Breast cancer is not a single disease but it is a disease with multiple distinct biological subtypes and varied natural history. It has a wide spectrum of clinical, pathological and molecular features which alter prognosis and management. Over the last few decades there have been outstanding advances in breast cancer management resulting in drastic decrease in mortality and decreased morbidity for women living with disease[5].

Stratification of patients after taking into consideration various prognostic parameters has assumed a great therapeutic importance[1]. Histopathological examination of the primary carcinoma and the axillary lymph nodes is a major prognostic indicator. Many prognostic and predictive factors have been identified by the College of American Pathologists to guide the clinical management of women with breast cancer. The prognostic factors includes invasive carcinoma or in-situ disease, distant metastases, lymph node metastases, tumour size, locally advanced

disease, histologic grade, histologic subtype, inflammatory carcinoma and hormonal receptor status and increased expression of HER2/neu[6].

In the current management guidelines, estrogen receptor (ER), progesterone receptor (PR) status and overexpression of HER2/neu are the three most useful predictive factors for response to specific therapeutic agents[4].

HER2/neu, otherwise known as neu or c-erbB-2, is the product of an oncogene. Its amplification and over expression varies from 11% to 32% of breast carcinomas[7]. Today Immunohistochemistry (IHC) is the centre stage in the demonstration of newer monoclonal antibodies[8]. IHC is used commonly for evaluating Her2/neu protein expression in breast cancer. Her2/neu expression is an independent prognostic factor in patients with both node- positive and node-negative breast cancer.

HER2/neu has greater prognostic value than most currently used prognostic factors including ER, PR status[9]. HER2/neu positive cancers exhibit resistance to tamoxifen but not to aromatase inhibitors or ovarian ablation[10].

The presence of HER2/neu is related to a high grade tumour and poor prognosis but favourable response to monoclonal antibody therapy and disease survival[11]. Therefore, there is a growing clinical demand for analysis of the Her2/neu status of current and archived breast cancer specimens[12].

Aims & Objectives

AIMS AND OBJECTIVES

1. Study of the HER2/neu status in breast carcinomas in the study sample.

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Review of Literature

REVIEW OF LITERATURE

History

The first reference to breast cancer dates back to "The Edwin Smith Surgical Papyrus" (3000-2500 BC), that mentions the diagnosis and treatment of eight cases of ailments of the breast. The author writes that there is no treatment for the breast tumours that are cool to touch and are bulging[13].

A detail account of breast cancer cases was described by Hippocrates. He described tumours in the breast became increasingly firm, with no pus. They eventually spread to other parts of the body, accompanied by shooting pain radiating from the breast to the neck and shoulder blades. The death was certain with the development of thirst and emaciation. He advised not to treat the hidden cancers[13].

The manuscript, „De Medicina", written by Celsus, defined four stages of breast cancer. The first stage was cacoethes (inflammation), followed by carcinoma without skin ulceration and later carcinoma with ulceration. The final stage was advanced exophytic and sometimes bleeding lesion, called the "thymium" which resembled the flowers of thyme. Celsus recommended excision for cacoethes and no treatment for other stages. In situations of uncertainty, the tumour was first treated with caustics and if symptoms improved, then it was cacoethes. If they worsened, then it was a carcinoma. Some masses for which treatment was successful might have been fibroadenomas, phyllodes tumours or even tuberculosis[13]. The nineteenth century witnessed remarkable advancements in human pathology and in the safety of surgery[13]

The advent of microscope by Anton Van Leuwenhoek (1674-1723) triggered the world of microscopic anatomy and Germany became the pioneer of „compound achromatic microscope"[13].

Johannes Muller (1801-1859) was the first to report that cancers also were composed of living cells[13].

The advancements in the surgical field brought modified mastectomies into existence. For the fear of spreading cancer with a biopsy, diagnosis was almost always clinical, established histologically only after the operation[13].

The discovery of x-rays provided the basis for radiotherapy and mammography[13]. The next 100 years resulted in a retreat from radical surgery with the introduction of mammography and chemotherapy. Breast cancer was recognized as a major health problem in the western world, stimulating a concerted effort against it[13].

Evolution of a prognostic index in breast carcinoma

Von Hansemann is credited for initiating many of the histological grading systems used today. He assessed the degree of nuclear anaplasia in tumours and noted that, the greater the degree of nuclear atypia, the greater is the likelihood of a metastasis occurring[14].

An association was found between the degree of differentiation and prognosis in patients with squamous carcinoma of the lip by Broders in 1920. Greenough subsequently developed a grading method for breast carcinoma that separated tumours into three histological grades based on histological and cytological features.

The features taken into account were the amount of tubule formation, the secretory activity of cells, the overall size of cells and nuclei, variation in the size of both cells and nuclei; nuclear hyperchromatism, and mitotic activity[14].

Greenough"s method was simplified by Patey and Scarff that considered only three factors namely the tubule formation, variation in nuclear size and shape and nuclear hyperchromatism[14].

In 1957, Bloom and Richardson introduced a numerical scoring system to the method described by Patey and Scarff[11]. It combined the details of cell morphology (nuclear pleomorphism) with a measurement of differentiation (tubule formation) and an assessment of proliferation (mitotic frequency) [15].

A large study of 2219 cases were systematically studied by Emad Rakha et al in 1957 and concluded that histological grade, as assessed by the Nottingham modification of Bloom Richardson histological grading system, provides a strong predictor of outcome in patients with invasive breast cancer and should be incorporated in breast cancer staging systems[16].

A great many prognostic factors in breast cancer have been described, but few when placed in multivariate analysis retain independent significance. Prognosis is multifactorially determined and the best discrimination is achieved by integrating independently significant factors. A widely used method of integration is the Nottingham Prognostic Index (NPI), first described in 1982. It is the only Index to have both intra- and inter-centre prospective validation[17].

The earlier studies divided patients into three NPI groups but Blamey et al (2007) recognized six groups: an Excellent Prognostic Group (EPG) with an observed NPI range of 2.08–2.4, Good Prognostic Group (GPG) 2.42 to 63.4; Moderate Prognostic Group I (MPG I) 3.42 to 64.4, Moderate Prognostic Group II (MPG II) 4.42 to 65.4, Poor Prognostic Group (PPG) 5.42 to 66.4 and Very poor Prognostic Group (VPG) > 6.5[17].

The WHO has adopted the NPI consisting of the tumour size, tumour grade and lymph node status[5].

Prognostic parameters in breast carcinoma

Prognostic factors, although not specific predictors of response to a therapy, is helpful for selection of appropriate treatment to patients with malignancies. Patients with an extremely good prognosis after tumour excision may not require noxious adjuvant therapies which themselves carry significant morbidity[18]. Conversely, patients with a poor prognosis may benefit from an aggressive adjuvant approach. Thus, identification of the prognostic features of an invasive breast carcinoma is particularly important as the disease has a markedly variable course[19]. A group of women with curable carcinomas who do not receive significant benefit from adjuvant therapy can be identified, while others will succumb relatively rapidly to the disease[20]. Because of this widely differing clinical outcome and because of the commonness of breast cancer, prognostic factors and, more recently, predictive factors in this malignant disease are probably among the most widely studied[18,21].

Many recent studies of possible prognostic factors in primary breast cancer have taken into account novel variables, either morphologically, immunohistochemically or biochemically, which at least experimentally are associated with invasion, metastasis, differentiation or growth rate of the tumour[18, 21].

The prognosis of breast carcinoma is related to a number of clinical and pathologic parameters. These are as follows:

1. Age of patient: Women younger than 50 years of age at the time of diagnosis have the best prognosis and is low in case of older women. Relative survival declines after the age of 50 years[1, 22].
2. BRCA1 status: A worse overall survival has been noted in breast carcinomas with BRCA1 mutation carriers in the absence of adjuvant therapy[1, 23].
3. Pregnancy and oral contraceptives: Carcinoma of the breast manifesting during pregnancy or lactation is generally aggressive tumour with low expression of hormone receptors and high expression of Her2/neu and is associated with poor prognosis. However, no convincing evidence has been found that prior use of oral contraceptive agents has an effect on the evolution or survival of breast carcinoma[1].
4. Early diagnosis: The relative survival rates for asymptomatic breast carcinomas are higher than those for clinically detectable carcinomas[1].
5. Presence or absence of invasiveness: Majority of women with adequately treated DCIS are cured. In contrast, at least half of the invasive carcinomas have metastasized locally or distantly at the time of diagnosis[4].

6. Size: There is a good correlation between the diameter of the primary tumour and the incidence of nodal metastasis and the survival rate[24]. The risk of axillary lymph node metastases increases with the size of primary tumour[1].

7. Histological type: The favourable prognosis of certain histologic types of invasive carcinoma of the breast is now well recognized. Thus, tubular carcinoma [25, 26, 27], mucinous carcinoma[28, 29], invasive cribriform carcinoma[30], medullary carcinoma[31, 32], infiltrating lobular carcinoma[33] and tubulo-lobular carcinoma[34] have been reported to have a more favourable prognosis than invasive duct carcinomas of no special type[18].

8. Histological grade: Histological grade is an important determinant of prognosis that allows risk stratification within a given tumour stage. Morphological assessment of the degree of differentiation provides useful prognostic information in breast cancer. It has been recommended that all invasive breast carcinomas should be graded and the grading system used must be specified in the report. Grading is not restricted to tumours of no special histological type but special types are also included[18]. The most commonly used grading system, the Nottingham modification of Scarff-Bloom-Richardson grading (NSBR grading), combines nuclear grade, tubule formation, and mitotic rate [24].

9. Type of margins: Tumours with infiltrating margins have a poorer prognosis than tumours with pushing margins[1].

10. Tumour necrosis: There is an increased incidence of lymph node metastases and

decreased survival rates associated with tumour necrosis, particularly if it is extensive. This feature is usually associated with tumours of high histologic grade[1].

11. Stromal reaction: Tumours with a presence of inflammatory reaction at the periphery have a higher degree of nodal metastases and presumably poorer prognosis[1].

12. Skin invasion: A decreased survival rate is seen in breast carcinomas with invasion of the overlying skin[1].

13. Nipple invasion: Invasion of nipple by carcinoma is associated with a higher incidence of axillary metastases[1].

14. Lymphatic tumour emboli: There is an increased risk of tumour recurrence if there is presence of tumour emboli in the lymphatic vessels[1].

15. Blood vessel emboli: There is a high correlation between the presence of tumour emboli in the blood vessels and tumour size, histological grade, tumour type, lymph node status, development of distant metastases and poor prognosis[1].

16. Axillary lymph node metastases: Lymph node staging provides very powerful prognostic information in breast cancer. It is now generally accepted that the staging should be based on histologic examination of excised nodes rather than clinical examination. Numerous studies have shown that patients who have histologically involved regional lymph nodes have a much poorer prognosis than those without nodal involvement. The greater the number and the level of lymph nodes involved, the poorer the prognosis[1].

17. Internal mammary lymph node metastases: There is decreased survival rate in

patients with involvement of internal mammary lymph nodes compared to those without the involvement of these nodes[1].

18. Distant metastases: A cure is unlikely once the distant metastases are present and is associated with poor prognosis[1].

Review of Histological grading

The morphologic appearance of tumours can be correlated with their degree of malignancy[18]. Nearly 70 years ago, Scarff and his colleagues revised Greenhoughs method. The NSBR grading was adopted by WHO[35]. Morphological assessment of the degree of differentiation provides useful prognostic information in breast cancer, so now histological grading has been accepted as a routine procedure due to problems perceived with reproducibility and consistency[15]. It has been recommended that all invasive breast carcinomas should be graded and the grading system used must be specified in the report. Most of the special types of breast carcinoma are associated with a favourable prognosis, but this is generally true only for tumours with low-grade cytology. However, medullary carcinoma has a better prognosis than what the grading would suggest[36].

Histological grade is an important prognostic marker that allows risk stratification within a given tumour stage[36]. There are several histological grading systems in use; some consider ductoglandular differentiation or tumour secretory state. Some score only nuclear and nucleolar characteristics and others use both duct formation and nuclear abnormalities[37]. The most commonly used grading system, the NSBR grading, combines nuclear grade, tubule formation, and mitotic

rate (Table-1). Histological grade provides prognostic information along with the stage of disease. Poorly differentiated tumours are known to pursue a more aggressive course than their well differentiated counterparts[33, 38, 39, 40].

The WHO has adopted the Nottingham Prognostic Index consisting of the tumour size, tumour grade and lymph node status[5].

Review of immunohistochemistry

Immunohistochemistry (IHC) or immunocytochemistry is a method for localizing specific antigens in tissues or cells based on antigen antibody recognition. It seeks to exploit the specificity provided by the binding of an antibody with its antigen at a light microscopic level. IHC has a long history, extending more than half a century from 1940, when Coons developed an immunofluorescence technique to detect corresponding antigens in frozen tissue sections. However, only since the early 1990s has the method found general application in surgical pathology. A series of technical developments led eventually to the wide range of IHC applications in use today. The enzymatic label (horseradish peroxidase), developed by Avrameas and by Nakane and colleagues, allowed visualization of the labeled antibody by light microscopy in the presence of a suitable colourogenic substrate system[41].

In Oxford, Taylor and Burns in 1974 developed the first successful demonstration of antigens in routinely processed formalin fixed paraffin-embedded (FFPE) tissues. Critical issue in the development of immunoperoxidase techniques was related to the need to achieve greater sensitivity. Greater sensitivity would facilitate staining of FFPE tissues from a simple one- step direct conjugate

method to multiple- step detection techniques such as the peroxidase antiperoxidase (PAP), avidin-biotin conjugate (ABC), and biotin streptavidin (B-SA) methods and would eventually lead to amplification methods (such as tyramide) and highly sensitive polymer- based labeling systems. As the IHC method has evolved, its use in diagnostic pathology has expanded such that the use of one or more IHC stains is routine in surgical pathology, especially with respect to tumour diagnosis and classification. IHC has also been adapted to the identification and demonstration of both prognostic and predictive markers[41].

Enzyme digestion was introduced by Huang as a pre-treatment to IHC staining to unmask some antigens that had been altered by formalin fixation. The antigen- retrieval (AR) technique, based on a series of biochemical studies by Fraenkel- Conrat and coworkers was developed by Shi et al in 1991. In contrast to enzyme digestion, the AR technique is a simple method that involves heating routinely processed paraffin sections at high temperature before IHC staining procedures. The intensity of IHC staining was increased dramatically after AR pre-treatment. Subsequently various modifications of the AR technique have been described. Worldwide application of AR- IHC in pathology has validated the feasibility of AR-IHC and expanded its use in molecular morphology, while raising some basic questions and practical issues that are subject to ongoing evolution of the method[42].

The "College of American Pathologists Consensus Statement" has defined three categories of prognostic markers. The Category I includes factors of proven histological importance and are useful in clinical management namely the TNM

staging, the histological grade, the histological subtype, the mitotic count and the hormone receptor status. These factors hence become mandatory inclusions for a breast pathology report. The category II factors are extensively studied biologically and clinically but their importance remains to be validated with more robust studies. The factors are Human epidermal growth factor receptor2/ neuroblastoma (HER2/neu), lymphovascular invasion, p53, proliferative markers (MIB-1). The category III factors are not yet sufficiently studied to prove their prognostic value. These include DNA ploidy, microvessel density, EGFR, Bcl2, pS2 and Cathepsin D[5,6].

Biologic markers in normal breast

Estrogen Receptor[43]:

- It can be demonstrated in the nuclei of both ductal and lobular epithelial cells, with a higher proportion in lobules than in ducts.
- However, even in the lobules, only a small proportion of the cells show ER immunoreactivity, thus leading to heterogeneity of ER expression.
- In breast tissue from premenopausal women, there is generally an inverse relationship between expression of ER and markers of cell proliferation.
- The ER positive cells do not show expression of the proliferation related antigen Ki-67, and the Ki-67-positive cells are typically ER- negative.
- The proportion of ER-positive cells gradually increases with age but remains relatively stable after the menopause.
- The incidence of lobules showing contiguous patches of ER1 positive cells also increases with age and with involutional changes.

- In premenopausal women, ER1 expression varies with the phase of the menstrual cycle, being higher in the follicular than in the luteal phase.
- Myoepithelial cells do not show ER immunoreactivity.
- Recent studies have indicated that a second form of ER, ER2, is also expressed in normal breast tissue.
- Expression of ER2 has been observed not only in epithelial cells of ducts and lobules, but also in myoepithelial cells, endothelial cells and stromal cells.
- The expression of this form of ER2 does not seem to vary with the phase of the menstrual cycle.
- It has been speculated that the relative levels of ER1 and ER2 may be important in determining the risk of breast cancer development.
- Higher levels of ER2 relative to ER1 are protective against neoplastic progression in the breast.
- However, additional studies are needed to more clearly elucidate the role of ER2 in normal breast physiology and in breast cancer pathogenesis.

Progesterone receptor (PR)

- Has not been as extensively studied in normal breast tissue as has ER.
- PR is expressed in the nuclei of ductal and lobular epithelium.
- PR expression does not seem to vary with the menstrual cycle phase.

Other biomarkers

- Expression of a wide variety of biomarkers has been studied in benign breast tissue.
- Rarely, normal breast epithelium may show Her2/neu protein overexpression,

p53 protein accumulation, or p53 mutations, but the clinical significance of these findings is uncertain.

- The anti-apoptotic protein bcl-2 is consistently expressed by normal breast epithelial cells.
- The S-100 protein is strongly expressed by normal myoepithelial cells and variably expressed by mammary epithelial cells.
- Epithelial cells also show variable expression for casein, lactalbumin, gross cystic disease fluid protein-15, and c-kit (CD117), among other proteins.
- The cytokeratins 7, 8, 18, and 19 are typically expressed by epithelial cells, whereas myoepithelial cells express cytokeratins 5, 6, 14, and 17.

Molecular prognostic markers in breast carcinoma

Immunohistochemical analysis of prognostic and predictive factors like ER, PR, Ki-67, Her2/neu and p53 are being increasingly employed by the pathologists.

Hormone receptors

The normal breast epithelium contains receptors for estrogen and progesterone. The interaction between these receptors and hormones stimulates the proliferation and differentiation of cells. About 60 – 70 % of breast carcinomas also express these receptors. The patients with estrogen receptor positive tumours have a longer survival rates than others[44].

HER- 2/neu

HER- 2/neu also known as c-erb B- 2, is another proto-oncogene product associated with reduced survival when it is amplified and overexpressed in breast

cancer[1]. HER2/neu overexpression is associated with poor survival, but its main importance isas a predictor of response to agents that target this transmembrane protein[4].

p53

The p53 is a tumour suppressor gene normally involved in suppression of the cell cycle. The p53 mutation and overexpression is seen in about 50% of breast cancers and are strongly associated with an increased tumour proliferation rate and poor clinical outcome[44].

Proliferation markers

Proliferation can be measured by mitotic counts (e.g., as part of histologic grading), by immunohistochemical detection of cellular proteins produced during the cell cycle (e.g., cyclins, Ki-67), by flow cytometry (as the S-phase fraction), or by thymidine labeling index. Carcinomas with high proliferation rates have a poor prognosis but may respond better to chemotherapy[4].

DNA content

The amount of DNA per tumour cell can be determined by flow-cytometric analysis or by image analysis of tissue sections. Tumors with a DNA index of 1 have the same total amount of DNA as normal diploid cells, although marked karyotypic changes may be present. Aneuploid tumours are those with abnormal DNA indices and have a slightly worse prognosis[4].

Telomerase activity

The level of this enzyme is associated with the proliferative index of breast carcinoma, but its measurement is not an independent predictor of survival[1].

Invasion related factors

Several enzymes, when expressed by tumour cells or by the adjoining stroma, promote local invasion or distant metastasis leading to decreased survival. These include recently identified factors such as laminin receptor, cathepsin D, stromolysin 3 and urokinase- plasminogen activator[44].

Eighty percent of carcinomas that are both ER and PR positive respond to hormonal manipulation, whereas only about 40% of those with either ER or PR alone respond. ER-positive cancers are less likely to respond to chemotherapy. Conversely, cancers that fail to express either ER or PR have a less than 10% likelihood of responding to hormonal therapy but are more likely to respond to chemotherapy[4].

Osborne et al in 1996 reviewed the literature and concluded that tumours with higher levels of estrogen receptor have a greater response rate than those with lower amounts. Response to hormonal therapy requires functioning receptors, so a marker that reflects the functional integrity of the pathway, rather than simply the existence of receptor protein, could help separate receptor-positive patients who will respond from those who will not[45].

Allen Gown in 2008 reviewed the literature and concluded that the presence of estrogen receptors (ERs), as detected by IHC, is a weak prognostic marker of clinical outcome in breast cancer, although a strong predictive marker for response, for example, to tamoxifen-based therapy[46].

Review of HER2/neu

HER2/neu is a 185 kDa glycoprotein with tyrosine kinase activity. It is also known as NEU, c-erbB- 2 or human epidermal growth factor receptor 2[11]. It is a member of the human epidermal growth factor receptor gene family. Cells transfected with Her2/neu acquire a more malignant phenotype, with stimulation of cell proliferation, invasion, and metastasis[47].

ErbB-2 is a transmembrane growth factor receptor belonging to the type I receptor tyrosine kinase family of proteins. It is related to epidermal growth factor receptor (EGFR) and has high homology with other members of the EGFR family, which include ErbB-1, ErbB-3 and ErbB-4. The gene is located on chromosome 17q21 and encodes a 185 kDa protein. It has 1255 amino acids and is a highly phosphorylated protein. Normally, ErbB-2 is expressed in a wide variety of tissues and is involved in normal growth, cell adhesion and development. ErbB-2 can both homodimerize and heterodimerize with other members of the EGFR family and activates a range of signal transduction pathways[48].

HER2/neu gene was first identified in rat neuroblastoma cell lines by Schubert et al in 1974[49]. The relationship of HER2/neu with breast cancer was first identified in 1987 by Vijver et al[50].

Almasri et al in 2005 studied 91 cases and concluded that HER2/neu is overexpressed in 24% of cases of breast carcinomas[7].

Rashed et al in 2007 studied 50 cases and concluded that HER2/neu is

overexpressed in 26% of cases of breast carcinomas[51].

Ayadi et al in 2008 studied 155 cases and concluded that HER2/neu is overexpressed in 18.1% of cases of breast carcinomas[52].

Azizun-nisa et al in 2008 studied 150 cases and concluded that HER2/neu is overexpressed in 24.7% of cases of breast carcinomas and concluded that Her2/neu over expression is seen with increasing tumour size and grade[53].

Mudduwwa et al in 2009 studied 151 cases and concluded that HER2/neu is overexpressed in 19.1% of cases of breast carcinomas[54].

Ambroise et al in 2011 studied 321 cases and concluded that HER2/neu is overexpressed in 27% of cases of breast carcinomas[55].

Ahmed et al in 2011 studied 137 cases and concluded that HER2/neu is overexpressed in 30.6% of cases of breast carcinomas[55].

Wolff et al in 2007 reviewed the literature and concluded that HER2/neu is overexpressed in 18% to 20% of cases of breast carcinoma[11].

Valérie Pawlowski et al in 2007 studied 365 cases and confirmed that HER2/neu expression is a marker of tumour aggressiveness in breast cancer[56].

Shinichi Tsutsui et al in 2002 followed-up 698 cases of carcinoma breast for 54 months and concluded that HER2/neu positivity is associated with worse prognosis (higher rate of recurrence and mortality)[57].

Tatjana Ivkovic et al in 2007 studied 120 cases and found a statistically

significant correlation between HER2/neu protein overexpression and large tumour size, high histological grade and increased proliferative index[58].

Alfred Carr et al in 2000 did a follow up study of 190 cases of carcinoma breast and concluded that HER2/neu oncogene is an independent prognostic indicator in subset of breast cancers that are at high risk of early recurrence, regardless of tumour grade, estrogen/progesterone receptor status and lymph node status. Patients amplifying the HER2/neu oncogene have a shorter disease-free survival than patients without the oncogene[59].

A multivariate analysis of 314 cases by Amanda H. McCann et al in 2000 indicated that the HER2/neu oncoprotein was an independent prognostic indicator for overall survival in breast carcinoma patients[60].

DJ Slamon et al studied 189 cases in 1987 and concluded that HER2/neu has greater prognostic value than most currently used prognostic factors including ER, PR status[9]. The erbB- 2 status was superior to ER as a prognostic factor in breast carcinoma as studied by Heikki et al in 2003[61].

There are various methods of testing HER2/neu expression. IHC and Western blot are used to detect protein overexpression. Southern blot, PCR (Polymerase Chain Reaction), FISH (fluorescence in- situ hybridization) and CISH (chromogenic in- situ hybridization) are used for gene amplification and Northern blot for mRNA[62].

Selvarajan et al[63] studied 41 cases in 2004 and Benöhr P et al[64] studied 15 cases in 2005 and concluded that HER2/neu status can be reliably assessed by

IHC technique if adequate quality control is assured. High level of concordance between FISH and IHC results (91%, P < .001) was seen[12].

Rani James et al in 2011 studied 207 cases and concluded that HER2/neu positive cancers exhibit resistance to tamoxifen but not to aromatase inhibitors or ovarian ablation (medical or surgical) [10]. Similar conclusions were obtained by Love et al in 2003 by analysing 282 cases[65]. HER2/neu positivity is associated with relative resistance to non anthracycline nontaxane containing chemotherapy and responds to anthracycline treatment. Trastuzumab, a humanized monoclonal antibody, improves response rates, time to progression and survival when used alone or in combination with chemotherapy and decreases the recurrence by 33%[11].

Moradi et al in 2008 studied 339 cases of breast cancer and concluded that HER2/neu over expression is significantly associated with higher tumour grade but not with stage[66].

Ludovini et al in 2007 found significant association of Her2/neu with high histological grade and concluded that Her2/neu is an independent predictive factor for shorter overall survival and disease free survival in invasive primary breast cancer. There was no statistically significant association with menopausal status, histological tumour type, tumour size, stage and lymph node involvement[67].

Lovekin et al in 1991 studied 782 patients and found that Her2/neu expression is associated with poorer prognosis[68].

Al- Moundhri et al in 2003 studied 72 cases and found no significant association of er2/neu with patient age, tumour size, tumour grade and lymph

node involvement[69].

Several techniques are available for the genetic testing of Her2/neu amplification. FISH is a reliable method. Semiquantitative measurement using IHC for the Her2/neu membrane receptor protein can also accurately predict gene amplification. FISH for Her2/neu has a higher failure rate and reagent cost than IHC, and it takes longer to carry out and interpret than IHC[12].

GUIDELINES FOR HER2/NEU TESTING ACCORDING TO ASCO-CAP GUIDELINES, 2007 TO 2011[11]

Specimen Handling Considerations for Her2/neu

- The time duration from tissue removal to fixation (cold ischemic time) should be short (ideally less than an hour).
- Fixation time: It is the time when tissue is placed in fixative to time alcohol is introduced on the tissue processor (includes the time to grossing and the time the cassettes in formalin are loaded on the tissue processor).
- The tissue bisection through the tumour should be done immediately before tissue is placed in neutral buffered formalin (NBF), especially if sample is obtained remotely.
- Required fixation time: It is >6 to <72 hours in 10% NBF.

Analytic requirements for Her2/neu testing

- The external controls should be used with each batch, including weakly positive controls for each analyte.

- The internal acinar elements should be reviewed on each case if they are present: The normal breast epithelium should be negative for Her2/neu.
- The control tissues should be stained as on slide controls with each test if possible.

Quality assurance requirements for Her2/neu assay

- All assays should be validated before offering the testing in the laboratory, using 40 known positive (including weakly positive) cases and 40 known negative cases.
- The assays should be revalidated if any major modification in the assay procedure is done, using the same number of cases.
- Each assay run should be monitored with batch controls.
- External proficiency testing should be engaged for assays.
- External or internal laboratory accreditation should be engaged depending on standards in the region.
- Careful review and appropriate intervention should be conducted.
- Assay should be verified when minor modifications occur, using 20 known positive (including weakly positive) cases and 20 known negative cases.

Interpretation guidelines

Her2/neu IHC category of 3+ has been refined to assure that 95% would be FISH amplified if tested; 30% of cells must show homogeneous dark circumferential staining (chicken-wire pattern).

Her2/neu FISH must be scored on the area of tumour defined by the pathologist. If IHC is performed, FISH should be scored in the area of highest IHC staining.

Exercise caution in interpreting Her2/neu IHC when the following apply

- Non- formalin fixatives (alcohol-based fixatives used for FNA)
- Excessive delay from collection to fixation
- Overfixed tissue — Friday cases (>48–72 hours in formalin)
- Inadequately fixed tissue (<6–8 hours NBF)
- Excessive artifacts- edge, crush, disruption, necrosis
- Decalcified blocks
- Overexpression in benign elements

Final reporting considerations for Her2/neu testing

- Her2/neu is more likely in high-grade and poorly differentiated tumours.
- Low-grade tumours typically are ER/PR positive and Her2/neu negative, including the following:
  - Classic infiltrating lobular carcinoma
  - Mucinous carcinoma
  - Tubular carcinoma

A test result that does not fit the histological picture suggests the following:

- The assay may be invalid
- Repeat testing may be indicated.

Molecular markers of future prospects[43]

- The evaluation of DNA, RNA, and protein expression using the modern tools of molecular biology, such as laser capture microdissection will greatly enhance our understanding of breast tumourigenesis and may even serve to redefine what constitutes "normal".
- For example, a number of studies have shown that histologically normal TDLUs can exhibit an abnormal genotype, characterized by loss of heterozygosity or allelic imbalance at various chromosomal loci.
- However, the significance of these genetic alterations in histologically normal breast tissue remains to be determined.
- Studies of normal breast tissue using these techniques will also help define the presence and nature of progenitor cells or stem cells and their role in breast development and carcinogenesis as well as patterns of gene and protein expression that distinguish normal from abnormal breast tissue and cells.

p53, Akt kinase, carbonic anhydrase IX, COX-2, e-cadherin, tumour DNA ploidy, hsp27, ps2, cathepsin D, metallothionein, VEGF, TGF beta, placental growth factor, hepatocyte growth factor, pleiotropin, PDGF, cyclin E are the ongoing research markers in carcinoma breast.

Steps in modified radical mastectomy

1. Patient in supine position with elevated right hemithorax.
2. Parts painted and draped.
3. Incision is made according to the location of tumor so that tumor is included within the incision.
4. Skin flaps raised above upto subclavius muscle, medially upto sternum, laterally upto anterior margin of lattisimus dorsi, inferiorly upto 3 to 4cm from the inferior mammary fold.
5. Breast parenchyma and pectoralis major fascia elevated from pectoralis major muscle.
6. Level I,II,III group of axillary lymph nodes is removed depending on the involvement.
7. Axillary vein, thoracodorsal neurovascular bundle nerve to serratus anterior and medial pectoral nerve is preserved.
8. Saline wash should be given.
9. Wound closed in layers.

Figure-1: Showing intraoperative picture of modified radical mastectomy where axillary vein, thoracodoral neurovascular bundle, nerve to serratus anterior are preserved. Pectoralis major and minor muscles are retracted.

Methodology

METHODOLOGY

Patients diagnosed as carcinoma breast, admitted in Department of General surgery,Govt. Royapettah Hospital during the period from November 2014 to august 2015 were studied.

Clinical data were obtained from patients‟ history, clinical examination, hospital records and requisition forms received in the department. The specimens were sent to the pathology department and subjected to adequate fixation using 10% NBF. After adequate fixation, examination of the specimen for gross details according to the protocol for the examination of specimens of patients with invasive carcinoma of breast would be done. Then representative tissue bits subjected for routine processing and paraffin embedding. Three to four micron thick sections taken from paraffin embedded blocks. These sections routinely stained with haematoxylin and eosin (H & E) and examined noting the findings as per proforma.

Procedure for H & E staining

- Deparaffinise in xylene – 2 changes – 5 minutes each.
- Wash in absolute alcohol – 1 change – 3 minutes.
- Wash in water for 3-5 minutes.
- Stain with haematoxylin for 5 minutes.
- Wash in water for bluing for 3-5 minutes.
- Dip in acid alcohol – 1dip
- Wash in water for 3-5 minutes.
- Stain with eosin for 1 – 2 dips.

- Wash in water for 1-2 dips.
- Dip in alcohol – 1dip.
- Blot, dry and mount in DPX.

The tumours were typed according to the WHO classification system. The Nottingham modification of Bloom Richardson grading system was used for grading (Table 1).

Table – 1 : Histological grading using NSBR system

Criteria	Score 1	Score 2	Score 3
Tubule formation	>75% of tumour	10-75% of tumour	<10% tumour
Nuclear pleomorphism	Minimal variation in size and shape of nuclei	Moderate variation an size and shape of nuclei	Marked variation in size and shape of nuclei
Mitotic count/10 HPF (diameter 0.44 mm)	0-5	6-10	>11

Grade	Score
Grade 1	3-5
Grade 2	6-7
Grade 3	8-9

Grade 1 carcinoma includes tumours with combined scores of 3- 5; grade 2 includes scores of 6 and 7; and grade 3 includes tumours with the scores of 8 and 9.

Each case was assessed considering important clinicopathological prognostic parameters like size of the tumour, histological grade, presence of necrosis, lymphovascular invasion, involvement of surgical margins, fibrosis, stromal reaction, involvement of the skin in the form of nipple and areola/Pagetoid spread

and metastases in the axillary lymph nodes.

The grading of mitosis was done in all the cases and was divided into 3 grades. The cases with 0- 5 mitoses were placed in grade 1, 6- 10 mitoses in grade 2 and > 11 mitoses in grade 3 (Table 2).

Table – 2 : Table showing grading of mitosis

Mitosis grade	Number of mitoses
Grade 1	0- 5
Grade 2	6- 10
Grade 3	> 11

Lymph node staging was done in the 50 cases of carcinoma breast studied. The cases with no lymph nodes were placed in stage N0, 1- 3 lymph nodes with metastases were placed in stage N1, 4- 9 lymph nodes with metastases in stage N2 and > 10 lymph nodes with metastases in stage N3.

Table – 3 : Table showing staging of lymph nodes

Lymph node staging	Number of positive
Stage N0	0
Stage N1	1-3
Stage N2	4- 9
Stage N3	10 or more.

The Nottingham Prognostic Index (NPI) was calculated as follows[17]:

NPI = lymph node stage (1-3) + grade (1-3) + maximum diameter (cm x 0.2).

The scoring of lymph node stage in NPI is as follows: No metastases in the lymph node- Score 1; metastases in 1- 3 lymph nodes- Score 2 and metastases in > 4 lymph

nodes- Score 3. The histologic grading used is the NSBR grading (Table-1).

After calculation of the NPI score, all the 50 cases of carcinoma breast were divided into six prognostic groups (Table 4).

Table – 4 : Table showing Nottingham Prognostic Index (NPI) groups

Prognostic group	NPI Score
Excellent prognostic group (EPG)	2.08 – 2.4
Good prognostic group (GPG)	2.42 – 3.4
Moderate prognostic group I(MPG I)	3.42 – 4.4
Moderate prognostic group II (MPG II)	4.42 – 5.4
Poor prognostic group (PPG)	5.42 – 6.4
Very poor prognostic group (VPG)	6.42 – 6.8

All the 50 cases were subjected to IHC study for Her2/neu from the representative areas of the tumour. The polymer based IHC kit of BioGenex RTU was used.

Procedure of IHC staining for Her2/neu staining

Cut the sections at approximately 2-3 microns thick.

Float them on to positive charged slides.

Incubate for 37º C for one day and further incubate at 58ºC for over- night.

Two changes of xylene of 15 minutes each for deparaffinization.

Two changes of absolute alcohol of 1 minute each for rehydration.

One change of 90% alcohol of 1 minute for rehydration.

One change of 70% alcohol of 1 minute for rehydration.

Wash in tap water for 10 minutes.

Rinse in distilled water for 5 minutes.

Antigen Retrieval by Heat using pressure cooker for 4 whistles

Cooling of the sections to room temperature.

Rinse in distilled water for 5 minutes.

Wash in TBS buffer (pH•7.6) two times for 5 minutes each.

Treatment with peroxide block for 10- 15 minutes to block endogenous peroxidase enzyme.

Wash in TBS buffer (pH•7.6) three times for 5 minutes each.

Treatment with power block for 15 minutes to block non specific reaction with the other tissue antigens.

Drain the excess power block.

Treatment with primary antibody for Her2/neu for 30- 60 minutes to identify the tumour markers by antigen- antibody reaction.

Wash in TBS buffer (pH-7.6) three times for 5 minutes each.

Treatment with SS enhancer for 20 minutes to enhance the reaction

Wash in TBS buffer (pH-7.6) three times for 5 minutes each.

Treatment with SS Polymer (secondary antibody) for 30 minutes to elongate chain and also to label the enzyme.

Wash in TBS buffer (pH - 7.6) three times for 5 minutes each to wash unbounded antibodies.

Treatment with DAB working solution for 5-8 minutes to give brown colour to the antigens.

Wash in TBS buffer (pH•7.6) three times for 5 minutes each.

Wash in tap water for 5 minutes.

Counter stain with Harris haematoxylin for 1 minute.

Wash in tap water for 5 minutes to wash excess stain.

Two changes of xylene of 15 minutes each for deparaffinization.

- Two changes of absolute alcohol of 1 minute each for dehydration.
- One change of 90% alcohol of 2 minutes for dehydration.
- Two changes of absolute alcohol of 2 minutes for dehydration.
- Clearing with xylene for 2 minutes. Mount with DPX.

Preparation of reagents

Antigen Retrieval Buffer Tris

EDTA Buffer: pH – 9.0

Tris Buffer - 6.05 grams

EDTA - 0.744 grams

Dissolve in 1000 ml of distilled water.

1) Wash Buffer

Tris Buffered Saline: pH– 7.6

Tris buffer - 0.6 grams

NaCl - 8 grams Dissolve

in 1000ml of Distilled water. Adjust pH with

1N Hcl.

Precautions to be taken while testing

Do not allow sections to dry at any stage of the staining procedure. Carry out the steps of incubation with antibody at 37ºC. Use appropriate controls for each antibody tested.

Table – 5 : Table showing assessment of Her2/neu protein overexpression

Staining pattern	**Score**	Her2/neu protein overexpression assessment
No staining is observed or membrane staining is observed in less than 10% of the tumour cells	0	Negative
A faint/barely perceptible membrane staining is detected in more than 10% of the tumour cells. The cells are only stained in part of their membrane	1+	Negative
A weak to moderate complete membrane staining is observed in more than 10% of the tumour cells	2+	Weakly positive
A strong complete membrane staining is observed in more than 30% (formerly 10%) of the tumour cells	3+	Strongly positive

A score of 2+ and 3+ Her2/neu were considered positive for immunostaining.

Correlation of Her2/neu expression with above mentioned important clinicopathological prognostic parameters and with NPI was done.

Plan of data analysis

The collected data was entered in Excel sheet and analysed using Epiinfo software and the descriptive statistics, Chi-square test, Student‟s t-test, McNemar‟s test and other applicable statistical tests were applied for the data as applicable. The p value of < 0.05 was considered statistically significant.

Results

RESULTS

In the present study, a total number of 50 cases of carcinoma breast were evaluated during this retrospective and prospective study conducted from November 2014 to august 2015 in the department of general surgery,govt. royapettah hospital.

History

In the present study, the age of the patients with carcinoma breast ranged from 20 to 80 years. Majority of the patients (70%) were in the age group of 40 to 59 years (Table 6, Figure 1).

Table – 6 : Table showing presenting history of patients with carcinoma breast

Age (years)	Number (%)
< 39	6(12)
40 – 59	35(70)
> 60	9(18)
Pain	
Absent	41(82)
Present	9(18)
Nipple Discharge	
Absent	45(90)
Present	5(10)
Menopause	
Premenopausal	14(28)
Postmenopausal	36(72)

Figure – 2 : Chart showing age ditribution of patients with carcinoma breast

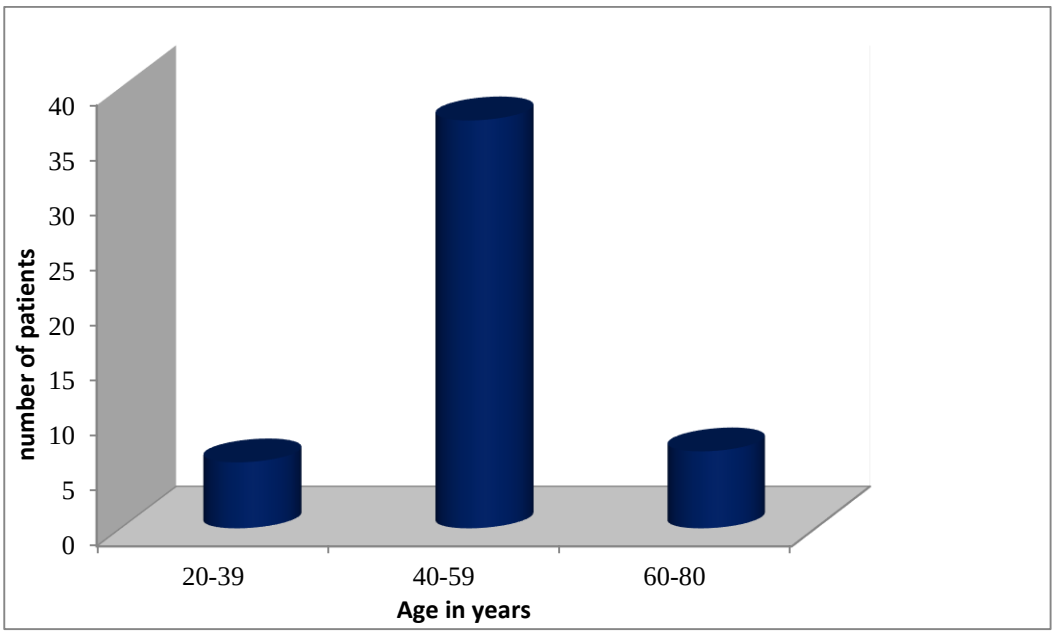


Figure-3: Chart showing pain in patients with carcinoma breast

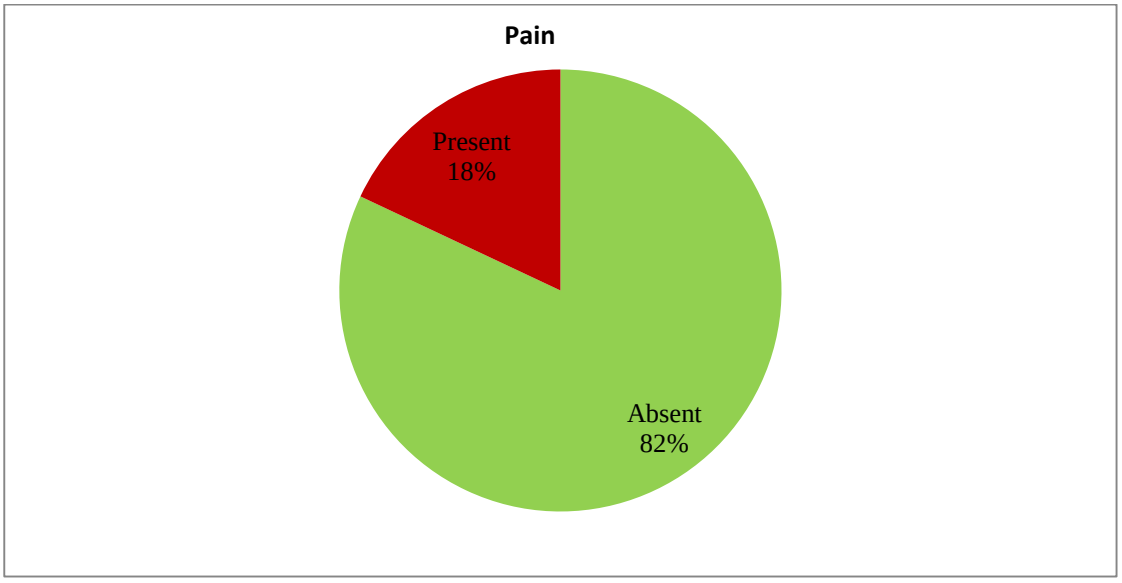


Figure-4:Chart showing nipple discharge in patients with carcinoma breast

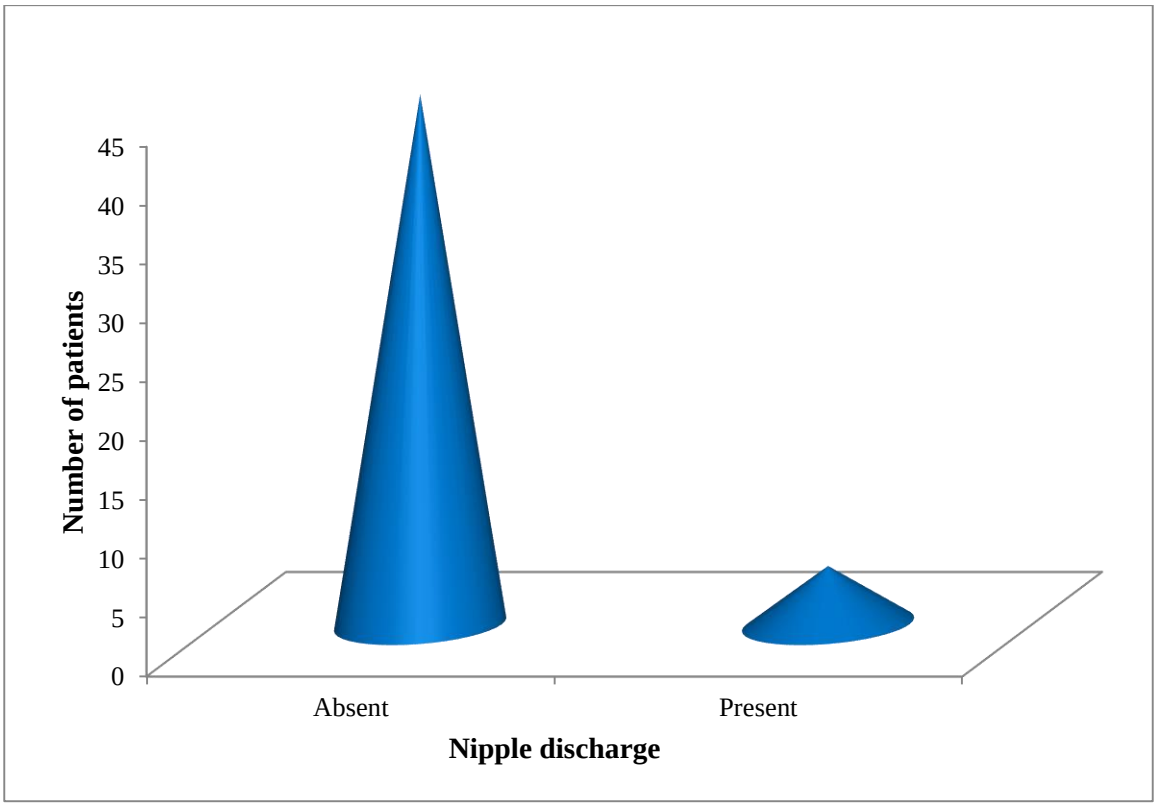
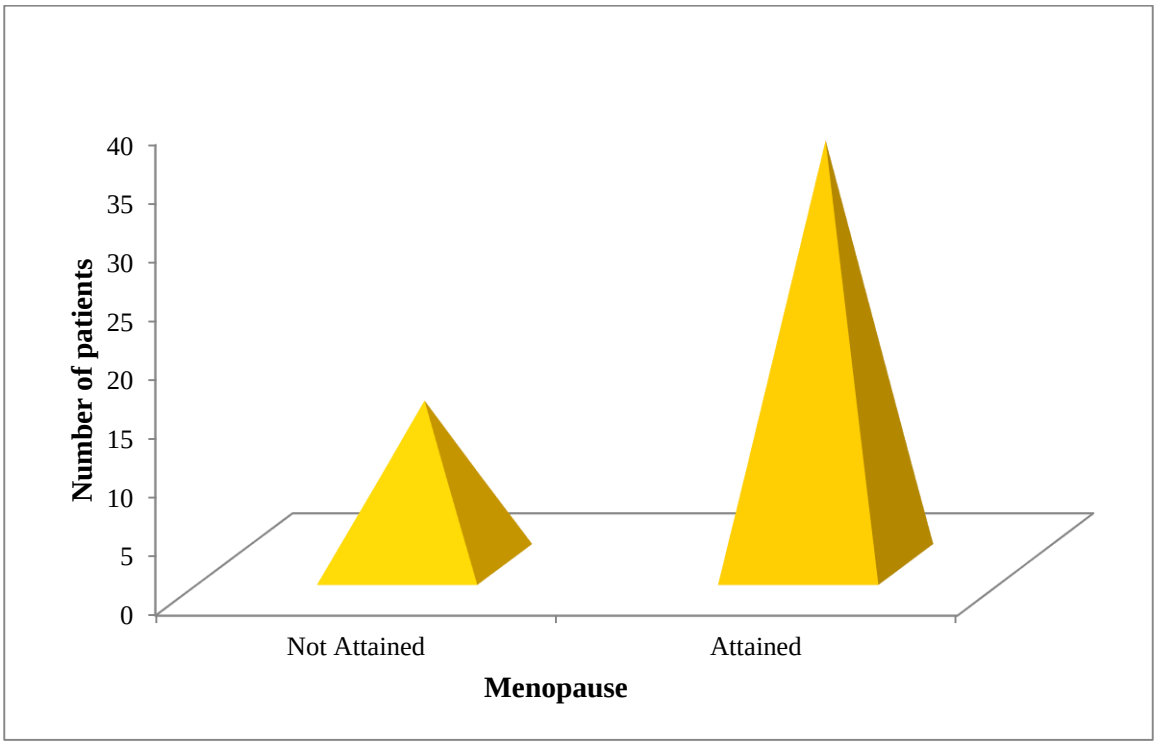


Figure-5:Chart showing menopausal status of patients with carcinoma breast



In the present study, all the 50 (100%) cases presented with lump in the breast, 9(18%) cases with pain in addition to lump (Table 6,Figure 3) and 5 (10%) cases presented with nipple discharge (Table 6, Figure 4).

In the present study 36 (72%) patients were in the premenopausal age group (Table 6, Figure 5).

Family history

In the present study, none of the cases had positive family history.

Past history

In the present study, none of the cases had past history of breast lesion.

Breast feeding

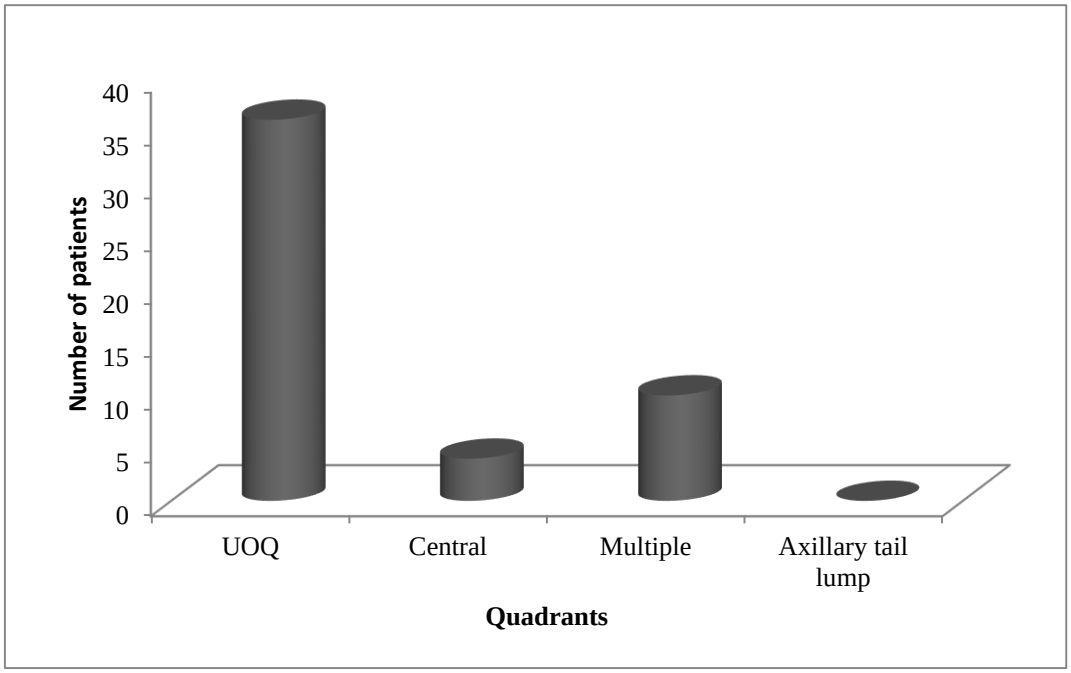
In the present study, 72% of the patients were multiparous and all of them had breast fed.

Clinical examination

Table – 7 : Table showing the distribution of carcinoma breast according to location of the tumour

Location of the tumour	Number (%)
UOQ	36 (72)
Central	04 (08)
Multiple	10 (20)
Axillary tail lump	00 (00)

Figure – 6 : Chart showing the distribution of carcinoma breast according to location of the tumor



On clinical examination, the tumour was in the upper outer quadrant (UOQ) in 36 (72%) cases. In 4 (8%) cases the tumour was central in location and in 10 (20%) cases the tumour was involving multiple quadrants (Table 7, Figure 6).

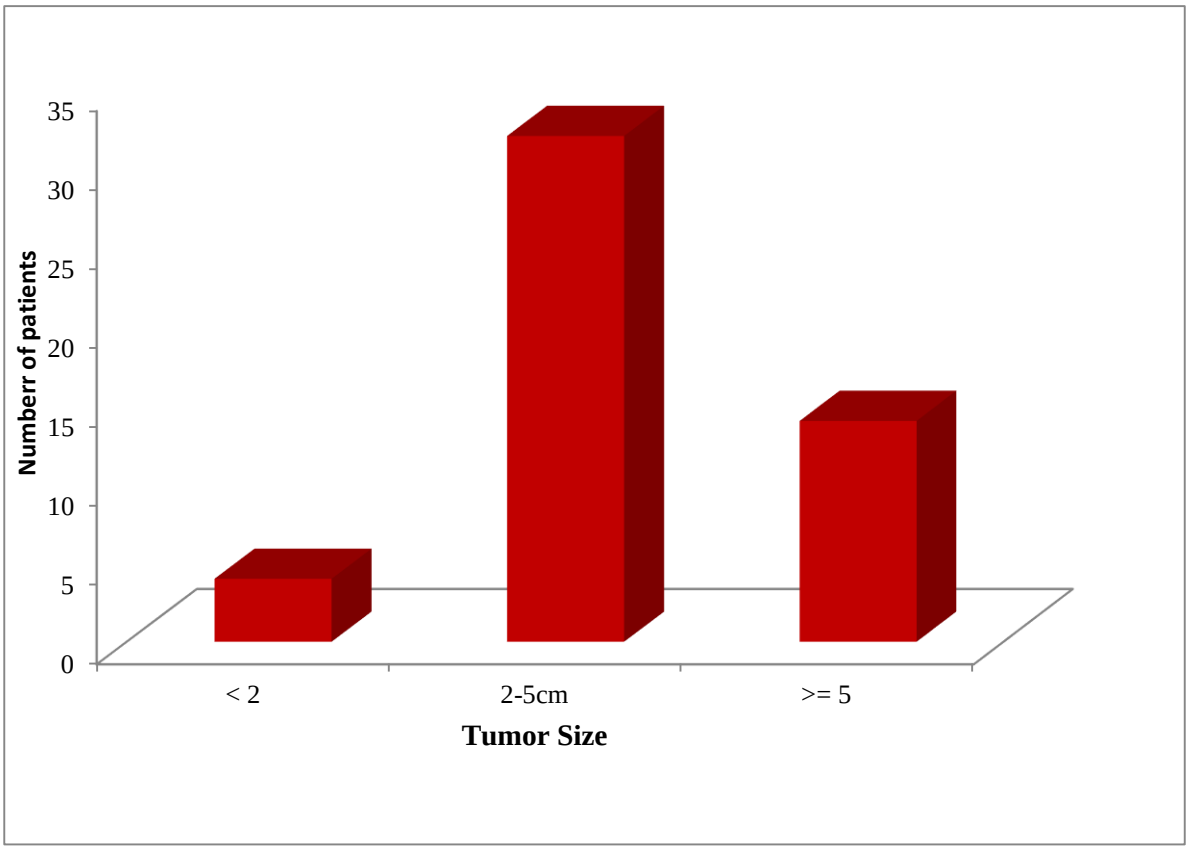
GROSS EXAMINATION OF SPECIMEN

All the specimens in the present study were modified radical mastectomy specimens. The relevant gross examination findings of the specimen were as follows

Table – 8 : Table showing the distribution of carcinoma breast according to size of the tumour

Tumor Size (cm)	Number (%)
< 2	4(8)
2 - 5	32(64)
≥ 5	14(28)

Figure – 7 : Chart showing the distribution of carcinoma breast according to size of the tumour

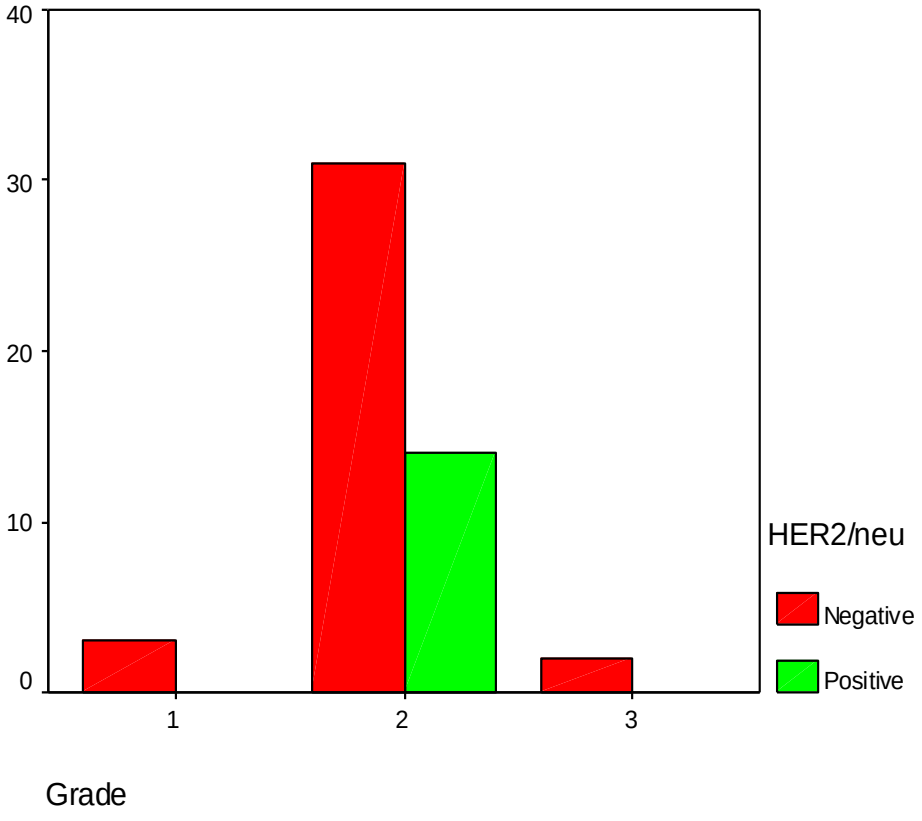


In the present study of 50 cases of carcinoma breast, the size of the tumour ranged from 1.5 cm to 8 cms considering the largest dimension of the tumour. In 4 (8%) cases the tumour size was < 2 cms, in 32 (64%) cases the tumour size ranged from 2 to 5 cms, and 14 (28%) cases had a of tumour size of > 5 cms (Table 8, Figure 7)

Table – 9 : Table showing the histological grading of the cases using Nottingham modification of Scarff Bloom Richardson (NSBR) system

NSBR Grade	Number (%)
1	3(6)
2	45(90)
3	2(4)

Figure – 8 : Chart showing the histological grading of the cases using Nottingham modification of Scarff Bloom Richardson (NSBR) system

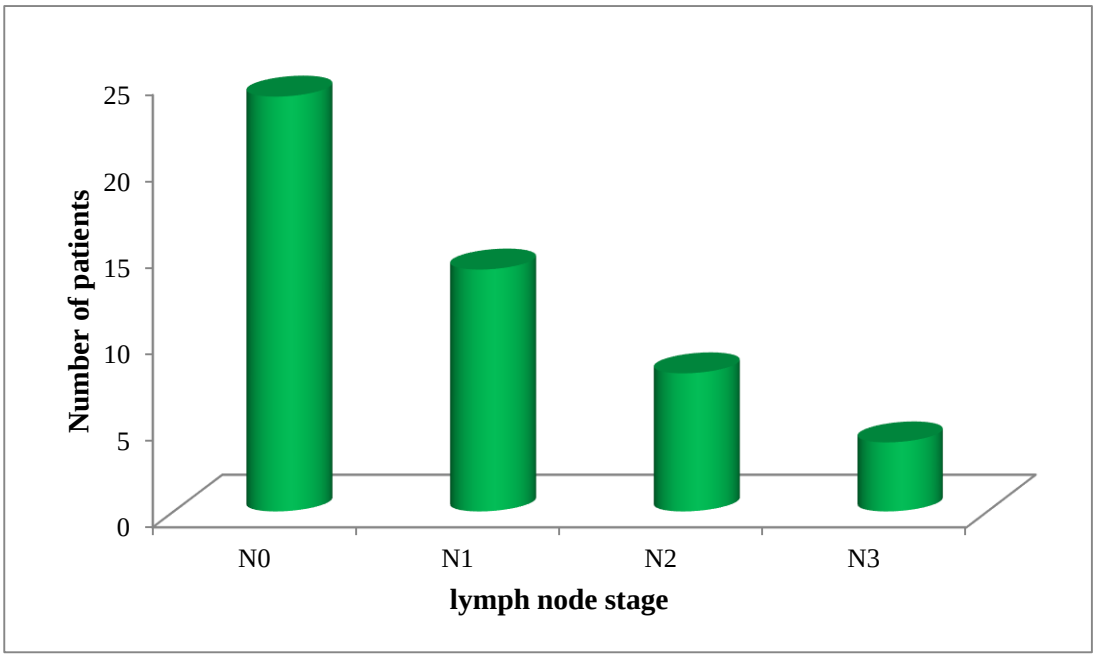


Of the 50 cases of invasive carcinoma breast studied, 3cases (6%) were of grade 1, 45 (90%) cases were of grade 2 and 2 cases (4%) belonged to grade 3 (Table 9, Figure 8).

Table – 10 : Table showing staging of lymph nodes

Positive lymph node	Number(%)
N0	24(48)
N1	14(28)
N2	8(16)
N3	4(8)

Figure-9:Chart showing staging of lymph nodes in carcinoma breast

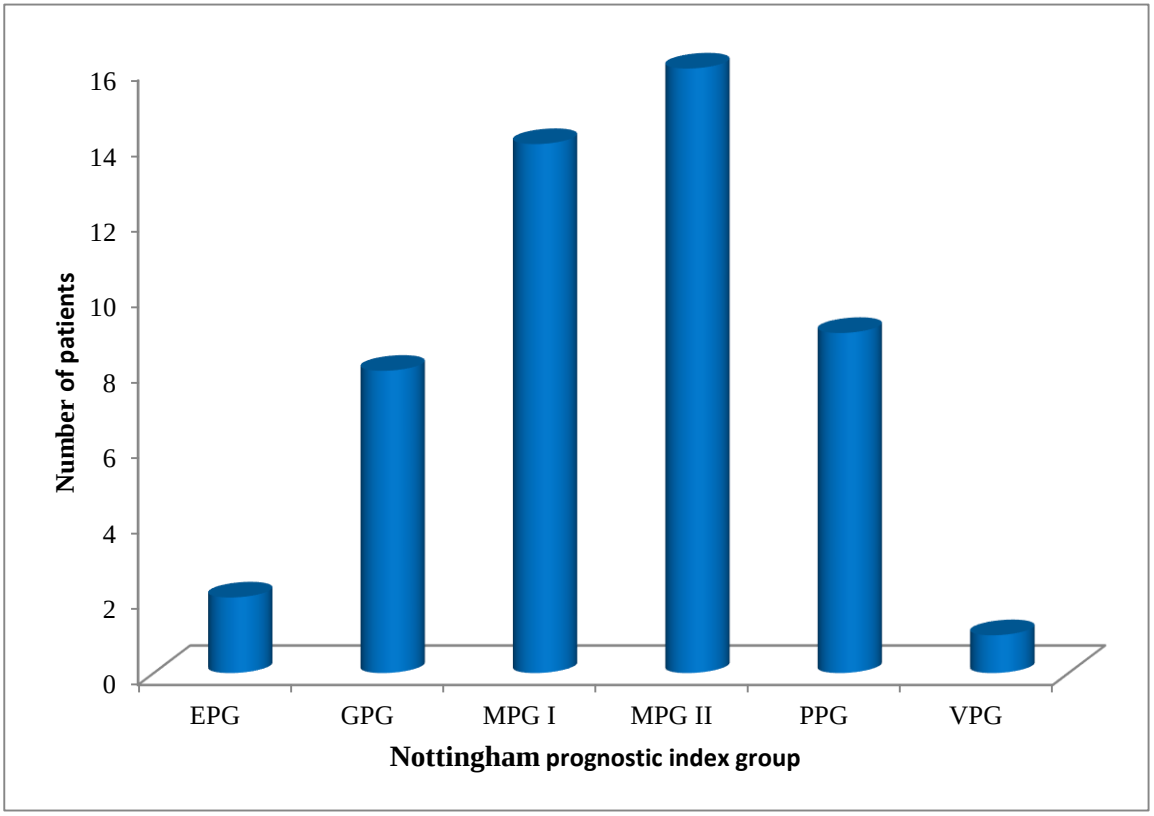


Of the 50 cases of carcinoma breast, 24 (48%) cases were of stage N0, followed by 14 (28%) cases of stage N1, 8 (16%) cases of stage N2 and 4 (8%) cases of stage N3 (Table 10,Figure 9).

Table – 11 : Table showing the distribution of carcinoma breast according to Nottingham prognostic index (NPI)

NPI group	Number (%)
EPG	2(4)
GPG	8(16)
MPG I	14(28)
MPG II	16(32)
PPG	9(18)
VPG	1(2)

Figure – 10 : Chart showing the distribution of carcinoma breast according to Nottingham prognostic index (NPI)



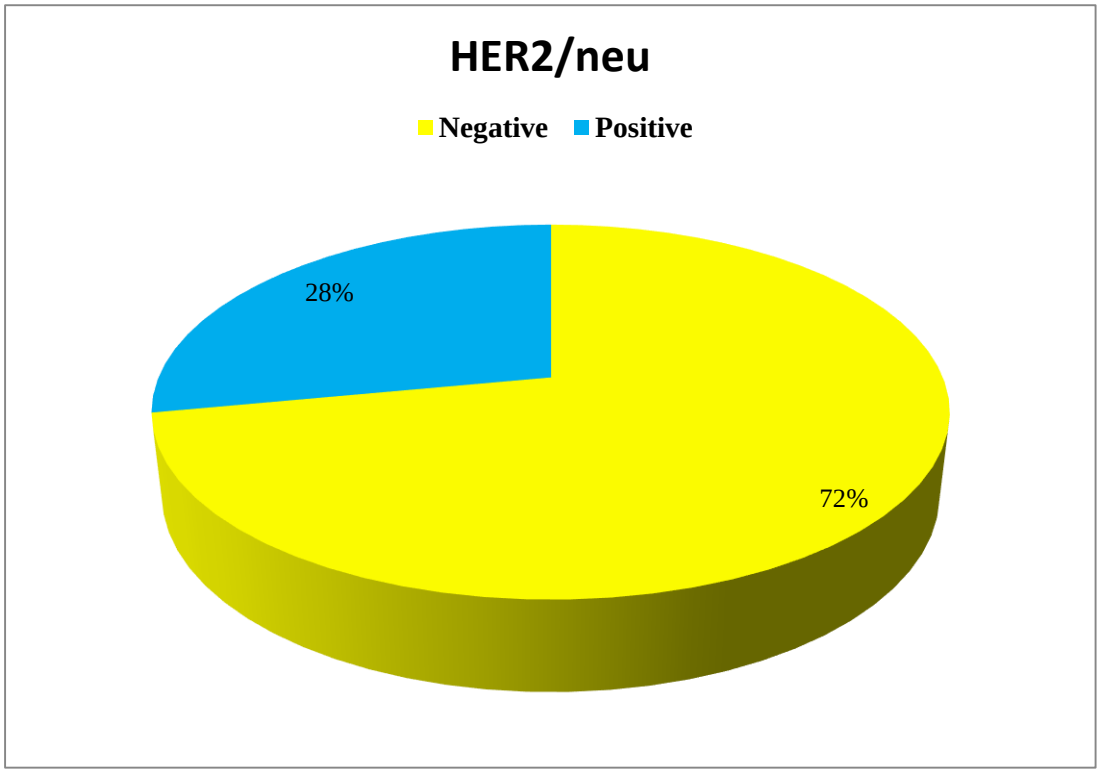
The NPI in the 50 cases of carcinoma breast showed 2 (4%) cases in EPG, 8(16%) cases in GPG, 14 (28%) in MPG I, 16 (32%) in MPG II, 9 (18%) cases in PPG and 1 (2%) in VPG groups (Table 11, Figure 10).

Immunohistochemistry

Table – 12 : Table showing Her2/neu expression of the tumour

Her2/neu expression	Number (%)
Negative	36(72)
Positive	14(28)

Figure – 11 : Chart showing Her2/neu expression of the tumour



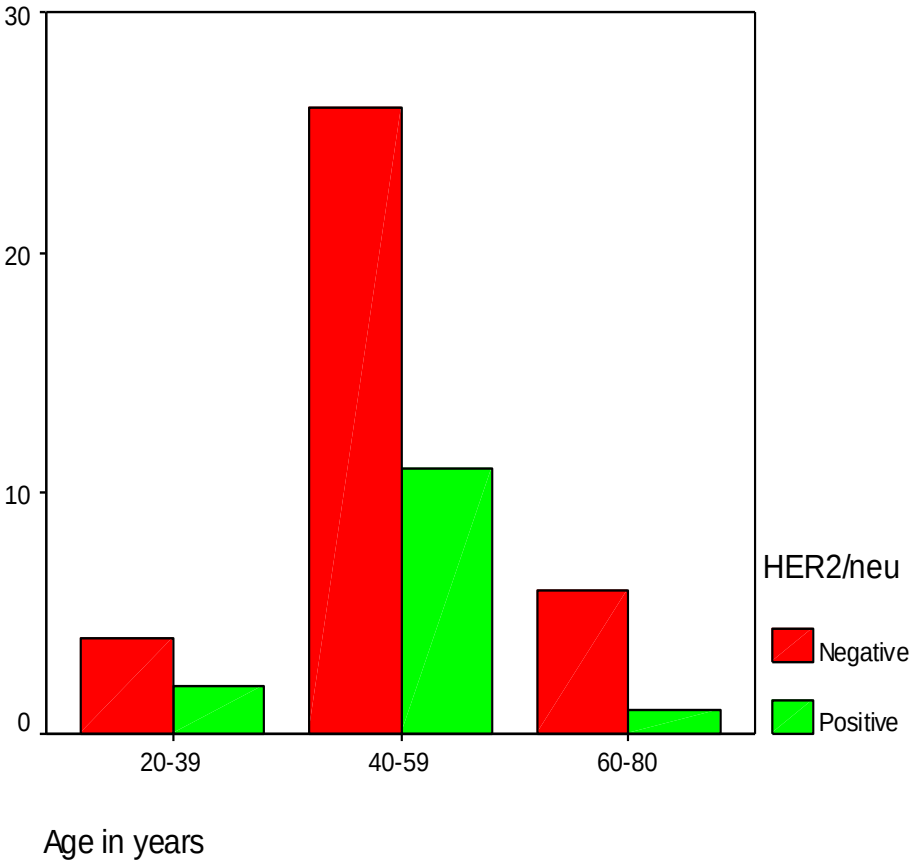
The Her2/neu was expressed in 14 (28%) out of 50 cases of carcinoma breast (Table 12, Figure 11) in the present study .

Association of Her2/neu with various clinicopathological and prognostic parameters

Table – 13: Table showing age distribution of carcinoma breast and its association with Her2/neu

Age in years	Number(%)	HER2/neu		Total	P value
		Negative	Positive		
20-39	Number	4	2	6	0.673
	% within Age in years	66.7%	33.3%	100.0%	
	% within HER2/neu	11.1%	14.3%	12.0%	
40-59	Number	26	11	37	
	% within Age in years	70.3%	29.7%	100.0%	
	% within HER2/neu	72.2%	78.6%	74.0%	
60-80	Number	6	1	7	
	% within Age in years	85.7%	14.3%	100.0%	
	% within HER2/neu	16.7%	7.1%	14.0%	
Total	Number	36	14	50	
	% within Age in years	72.0%	28.0%	100.0%	
	% within HER2/neu	100.0%	100.0%	100.0%	

Figure – 12 : Chart showing age distribution of carcinoma breast and its association with Her2/neu



Maximum Her2/neu positivity was seen in the age group of 40-59 years (78.6%) and the least Her2/neu positivity was seen between the age group of 60-80 years (7.1%) (Table 13, Figure 15). A statistically significant association of Her2/neu with age was not noted in the present study (p value = 0.673).

Table-14:Table showing pain in patients with carcinoma breast and its association with Her2/neu

Pain	Number(%)	HER2/neu		Total	P value
		Negative	Positive		
Absent	Count	32	9	41	0.042
	% within Pain	78.0%	22.0%	100.0%	
	% within HER2/neu	88.9%	64.3%	82.0%	
Present	Count	4	5	9	
	% within Pain	44.4%	55.6%	100.0%	
	% within HER2/neu	11.1%	35.7%	18.0%	
Total	Count	36	14	50	
	% within Pain	72.0%	28.0%	100.0%	
	% within HER2/neu	100.0%	100.0%	100.0%	

Figure-13:Chart showing pain in patients with carcinoma breast and its association with Her2/neu

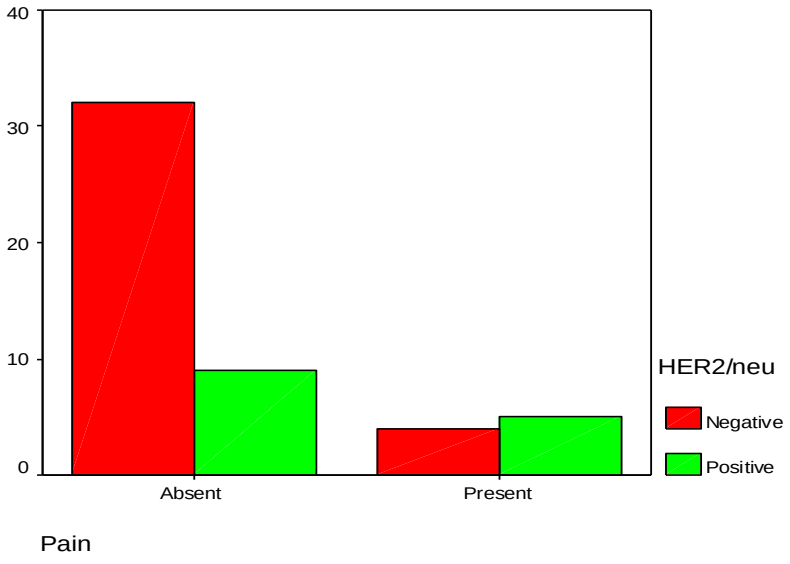


Table-15:Table showing nipple discharge in patients with carcinoma breast and its association with Her2/neu

Nipple dicharge	Number(%)	HER2/neu		Total	P value
		Negative	Positive		
Absent	Count	35	10	45	.006
	% within Nipple Discharge	77.8%	22.2%	100.0%	
	% within HER2/neu	97.2%	71.4%	90.0%	
Present	Count	1	4	5	
	% within Nipple Discharge	20.0%	80.0%	100.0%	
	% within HER2/neu	2.8%	28.6%	10.0%	
Total	Count	36	14	50	
	% within Nipple Discharge	72.0%	28.0%	100.0%	
	% within HER2/neu	100.0%	100.0%	100.0%	

Figure-14 : Chart showing nipple discharge in patients with carcinoma breast anditsassociation with Her2/neu

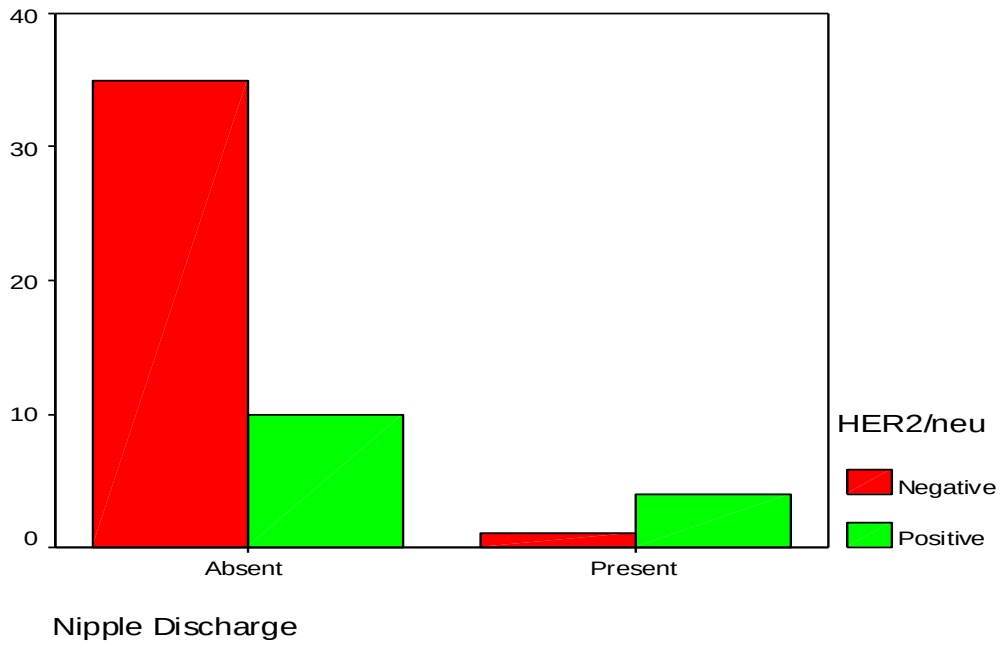
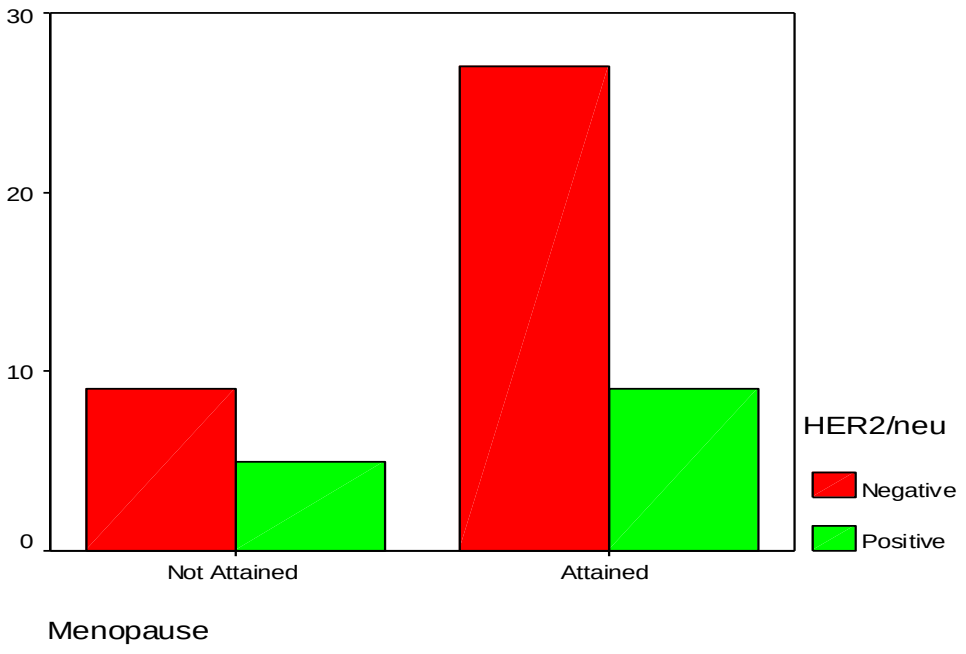


Table – 16: Table showing menopausal status of carcinoma breast and its correlation with Her2/neu

Menopause	Number(%)	HER2/neu		Total	P value
		Negative	Positive		
Not Attained	Count	9	5	14	0.449
	% within Menopause	64.3%	35.7%	100.0%	
	% within HER2/neu	25.0%	35.7%	28.0%	
Attained	Count	27	9	36	
	% within Menopause	75.0%	25.0%	100.0%	
	% within HER2/neu	75.0%	64.3%	72.0%	
	Count	36	14	50	
	% within Menopause	72.0%	28.0%	100.0%	
	% within HER2/neu	100.0%	100.0%	100.0%	

Figure – 15: Chart showing menopausal status of carcinoma breast and its association with Her2/neu

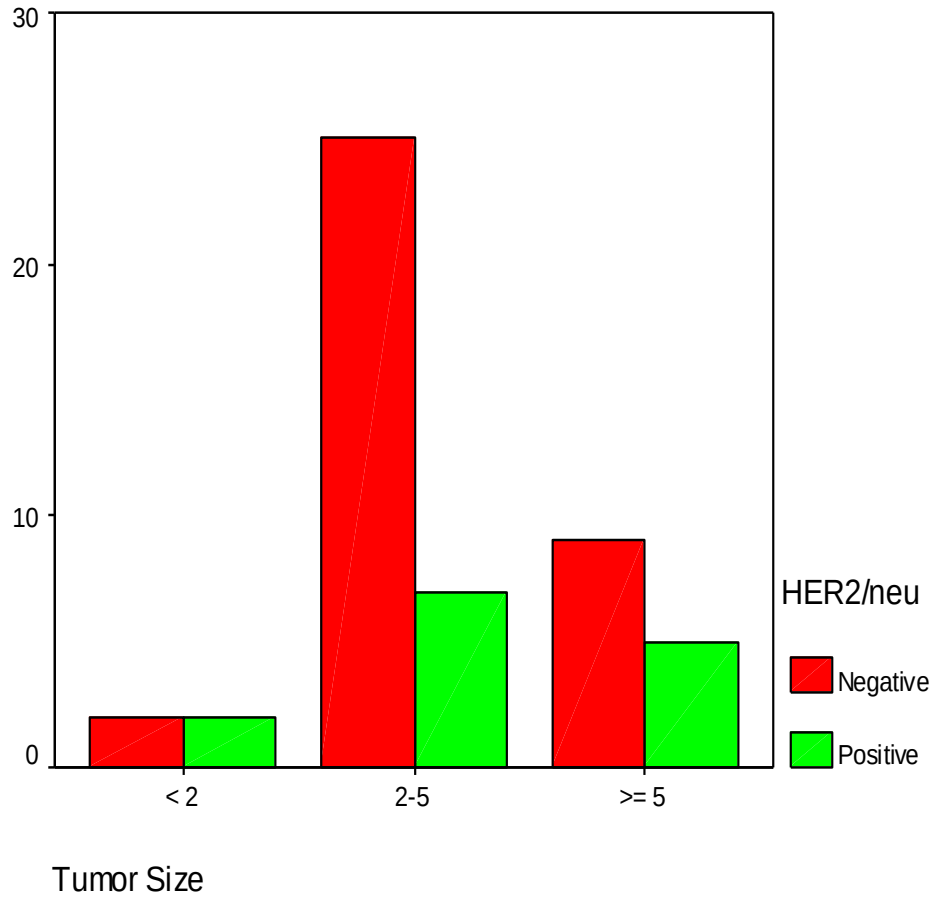


In the present study majority of patients (72%) were in the postmenopausal age group. Maximum Her2/neu positivity (64.3%) was seen in the post- menopausal age group (Table 16, Figure 15). No statistically significant association of Her2/neu with menopausal status was noted in the present study (p value = 0.449).

Table – 17 : Table showing size of the tumour and its association with Her2/ neu

Tumor size	Number(%)	HER2/neu		Total	P value
		Negative	Positive		
< 2	Count	2	2	4	0.374
	% within Tumor Size	50.0%	50.0%	100.0%	
	% within HER2/neu	5.6%	14.3%	8.0%	
2-5	Count	25	7	32	
	% within Tumor Size	78.1%	21.9%	100.0%	
	% within HER2/neu	69.4%	50.0%	64.0%	
>= 5	Count	9	5	14	
	% within Tumor Size	64.3%	35.7%	100.0%	
	% within HER2/neu	25.0%	35.7%	28.0%	
Total	Count	36	14	50	
	% within Tumor Size	72.0%	28.0%	100.0%	
	% within HER2/neu	100.0%	100.0%	100.0%	

Figure – 16 : Chart showing size of the tumour and its association with Her2/neu

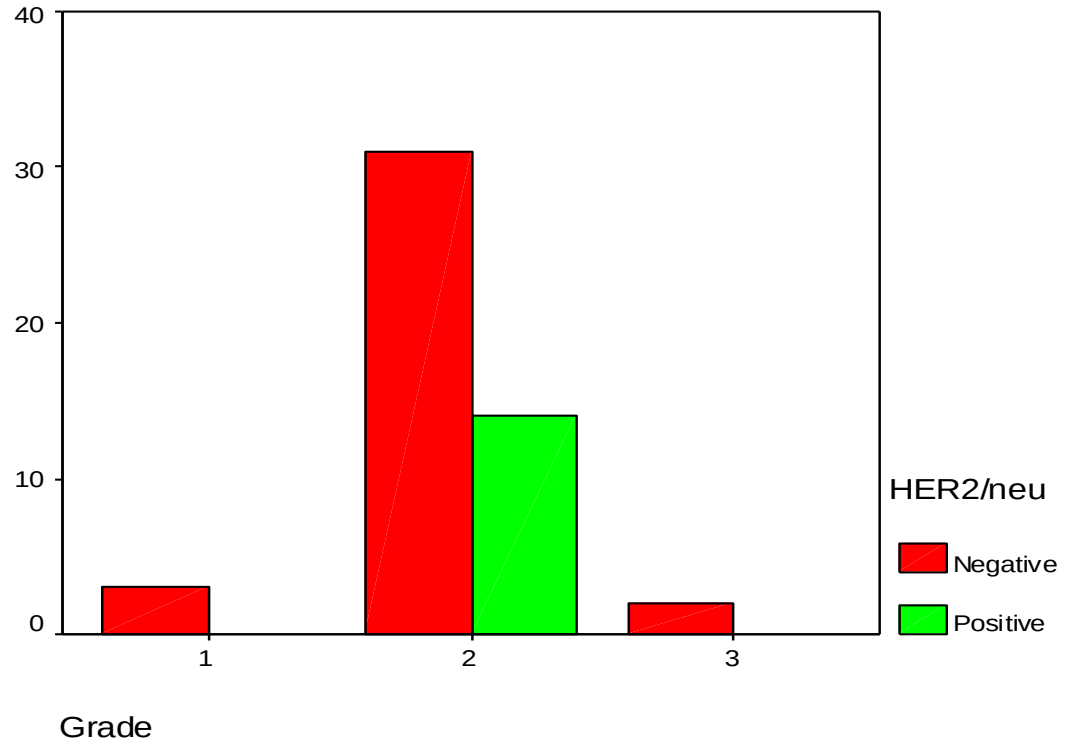


Cases with the tumour size of <2 cms were 2 cases negative and 2 cases positive for Her2/ neu. The maximum Her2/neu positivity was seen in 7(50%) with the tumour size between 2- 5 cms. Her 2/ neu was positive in 5 (35.7%) out of 14 cases with the tumour size > 5 cms (Table 17, Figure 16). No statistically significant association of Her2/neu with tumour size was noted in the present study (p value = 0.374).

Table -18: Table showing histological grading of tumour using Nottingham modification of Scarff Bloom Richardson system and its association with Her2/neu

Grade	Number(%)	HER2/neu		Total	P value
		Negative	Positive		
1	Count	3	0	3	0.340
	% within Grade	100.0%	.0%	100.0%	
	% within HER2/neu	8.3%	.0%	6.0%	
2	Count	31	14	45	
	% within Grade	68.9%	31.1%	100.0%	
	% within HER2/neu	86.1%	100.0%	90.0%	
3	Count	2	0	2	
	% within Grade	100.0%	.0%	100.0%	
	% within HER2/neu	5.6%	.0%	4.0%	
Total	Count	36	14	50	
	% within Grade	72.0%	28.0%	100.0%	
	% within HER2/neu	100.0%	100.0%	100.0%	

Figure - 17: Chart showing histological grading of tumour using Nottingham modification of Scarff Bloom Richardson system and its association with Her2/neu

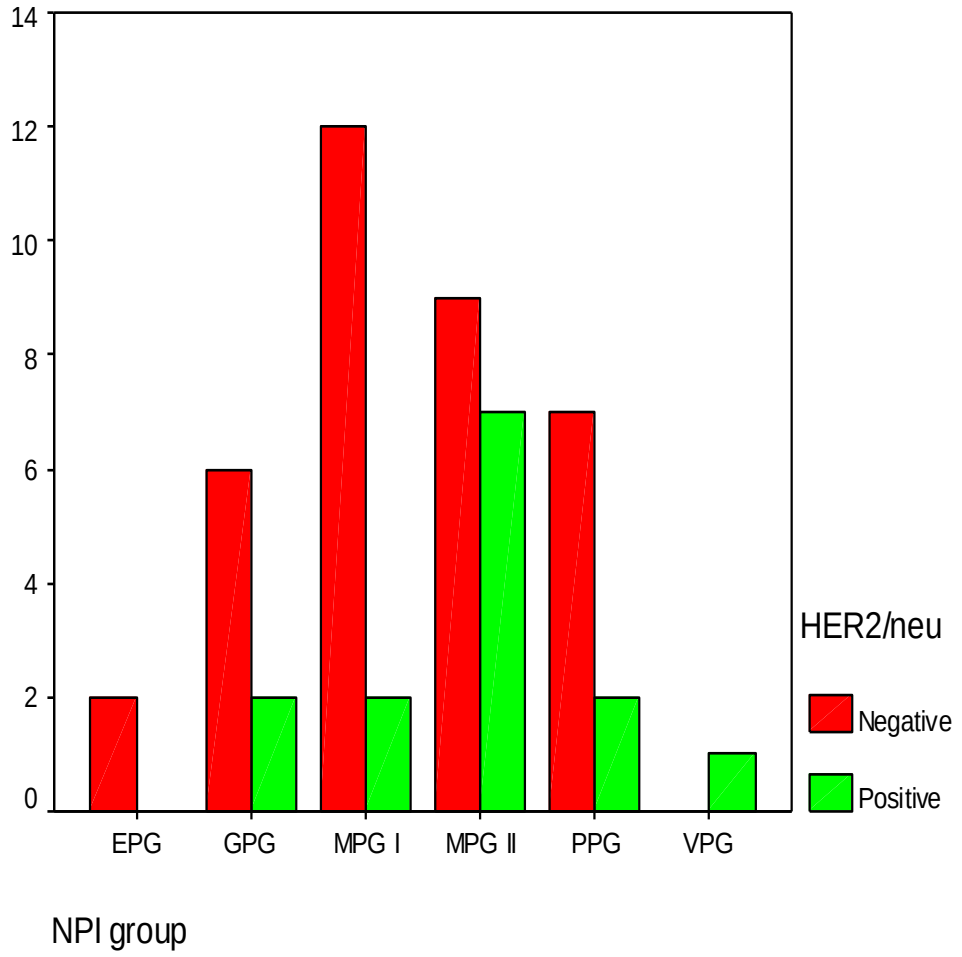


While correlating the over expression of Her2/neu with the histological grading, all cases (3 cases) of grade 1 tumours were negative for Her2/ neu, 68.9% (31 out of 45 cases) of grade 2 tumours and 100% (2 cases) of grade 3 tumours were negative for Her2/neu (Table 18, Figure 17). All cases of Her2/neu positivity were seen only in grade 2. No statistically significant association of Her2/neu with histologic grade was noted in the present study (p value = 0.340).

Table – 19: Table showing Nottingham Prognostic Index (NPI) and its correlation with Her2/n

NPI group	Number(%)	HER2/neu		Total	P value
		Negative	Positive		
EPG	Count	2	0	2	0.235
	% within NPI group	100.0%	.0%	100.0%	
	% within HER2/neu	5.6%	.0%	4.0%	
GPG	Count	6	2	8	
	% within NPI group	75.0%	25.0%	100.0%	
	% within HER2/neu	16.7%	14.3%	16.0%	
MPG I	Count	12	2	14	
	% within NPI group	85.7%	14.3%	100.0%	
	% within HER2/neu	33.3%	14.3%	28.0%	
MPG II	Count	9	7	16	
	% within NPI group	56.3%	43.8%	100.0%	
	% within HER2/neu	25.0%	50.0%	32.0%	
PPG	Count	7	2	9	
	% within NPI group	77.8%	22.2%	100.0%	
	% within HER2/neu	19.4%	14.3%	18.0%	
VPG	Count	0	1	1	
	% within NPI group	.0%	100.0%	100.0%	
	% within HER2/neu	.0%	7.1%	2.0%	
Total	Count	36	14	50	
	% within NPI group	72.0%	28.0%	100.0%	
	% within HER2/neu	100.0%	100.0%	100.0%	

Figure – 18: Chart showing Nottingham Prognostic Index (NPI) and its correlation with Her2/neu

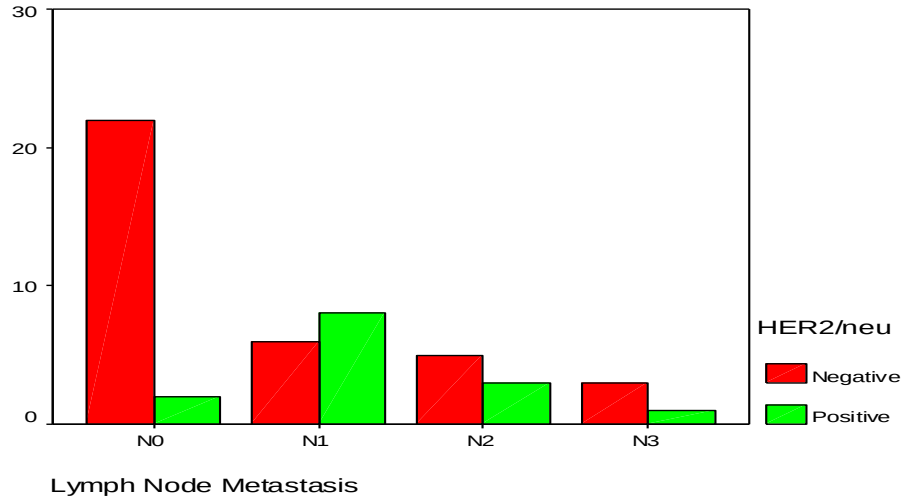


All the cases belonging to EPG were negative for Her2/ neu. The positivity of Her2/neu was seen in 2 (25%) cases of GPG, 2 (14.3%) cases of MPG I, 7 (43.8%) cases of MPG II, 2 (22.2%) cases of PPG and 1 (100%) cases of VPG prognostic groups (Table 19, Figure 18). No statistically significant association of Her2/neu with NPI was noted in the present study (p value = 0.235).

Table – 20 : Table showing Lymph node staging and its association with Her2/ neu

Lymph Node Metastasis	Number(%)	HER2/neu		Total	P value
		Negative	Positive		
N0	Count	22	2	24	0.012
	% within Lymph Node Metastasis	91.7%	8.3%	100.0%	
	% within HER2/neu	61.1%	14.3%	48.0%	
N1	Count	6	8	14	
	% within Lymph Node Metastasis	42.9%	57.1%	100.0%	
	% within HER2/neu	16.7%	57.1%	28.0%	
N2	Count	5	3	8	
	% within Lymph Node Metastasis	62.5%	37.5%	100.0%	
	% within HER2/neu	13.9%	21.4%	16.0%	
N3	Count	3	1	4	
	% within Lymph Node Metastasis	75.0%	25.0%	100.0%	
	% within HER2/neu	8.3%	7.1%	8.0%	
Total	Count	36	14	50	
	% within Lymph Node Metastasis	72.0%	28.0%	100.0%	
	% within HER2/neu	100.0%	100.0%	100.0%	

Figure – 19 : Chart showing Lymph node staging and its correlation with Her2/neu



Out of 50 cases of carcinoma breast, 24 (48%) showed no lymph node metastases in which only 2 (8.3%) out of 24 cases showed positivity for Her2/neu in the primary breast tumour.

The other 26 cases (52%) showed lymph node metastases with 14 **(**28%), 8 **(**16%) and 4 **(**8%) cases in stage N1, N2 and N3 respectively. Her2/neu was positive in 8**(**57.1%), 3**(**37.5%), and 1**(**25%) cases of stage N1, N2 and N3 respectively (Table- 20, Figure 19). Statistically significant association of Her2/neu with lymph node staging was noted in the present study (p value = 0.012).

Figure – 20 : Mastectomy specimen – Cut section showing gray white tumour of more than 5 cms

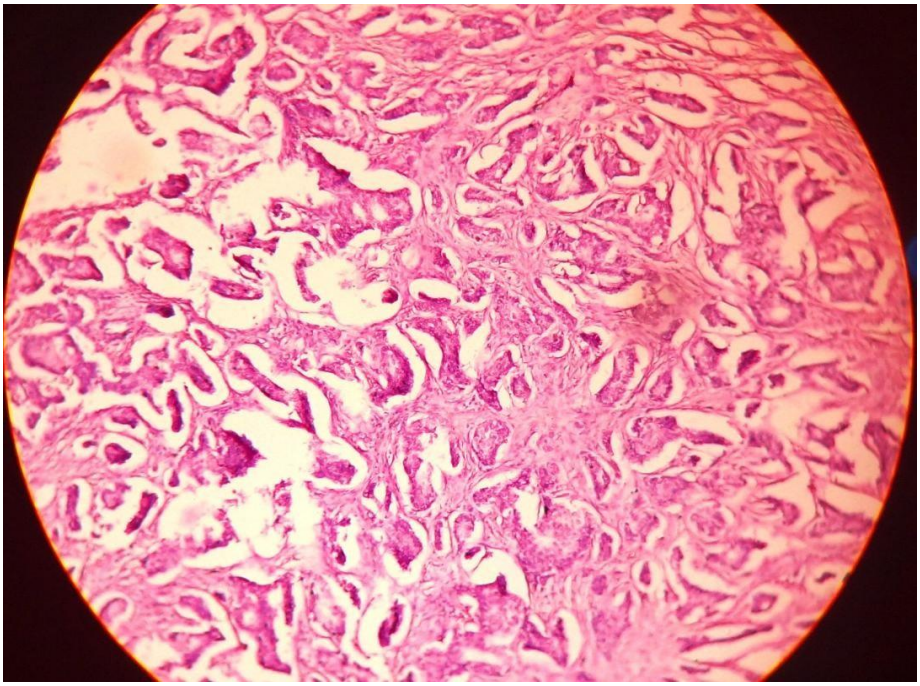


Figure – 21 : Microphotograph of Infiltrating duct carcinoma, NOS type (H & E, X50)

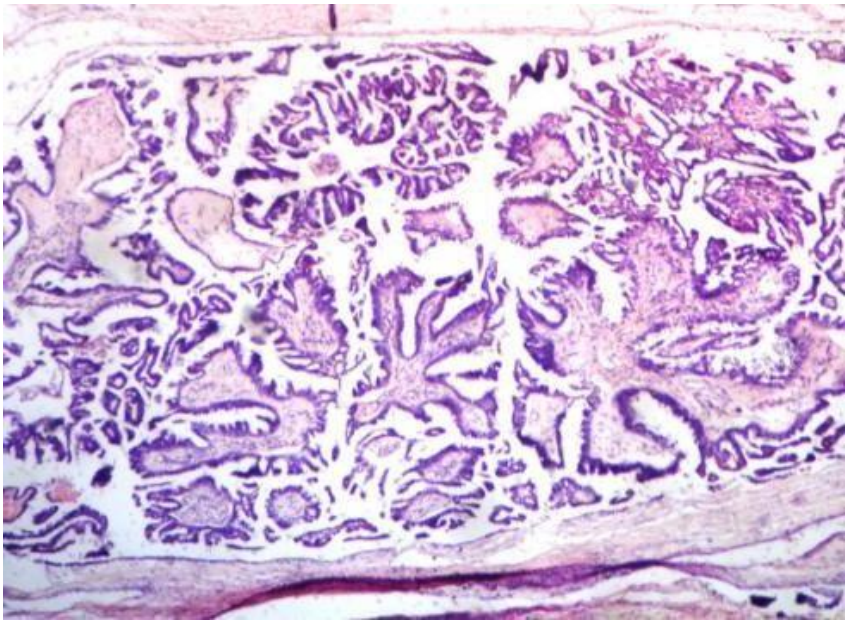


Figure – 22 : Papillary carcinoma (H & E, X 50)

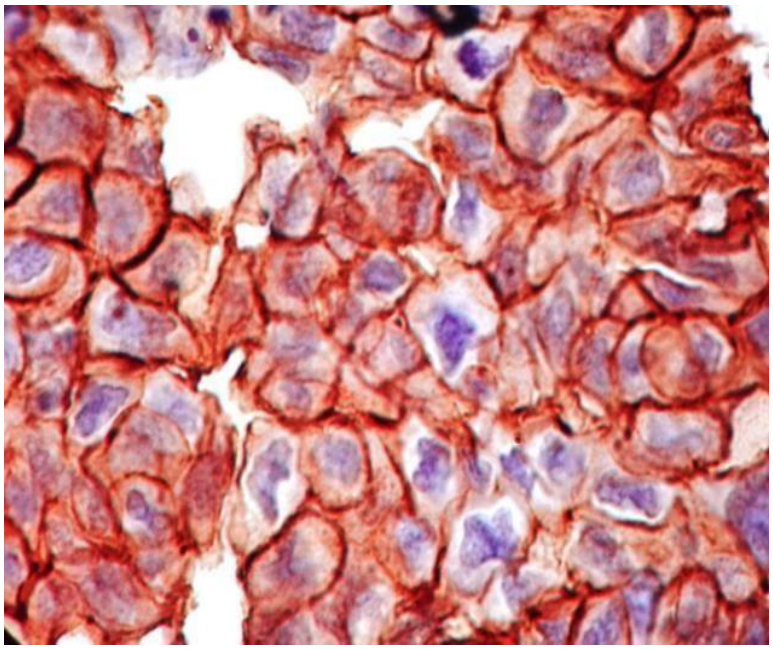


Figure – 24: Strong, complete membranous immunostaining (score of 3+) with HER-2/neu antibody in more than 10% of IDC cells (DAB, ×400)

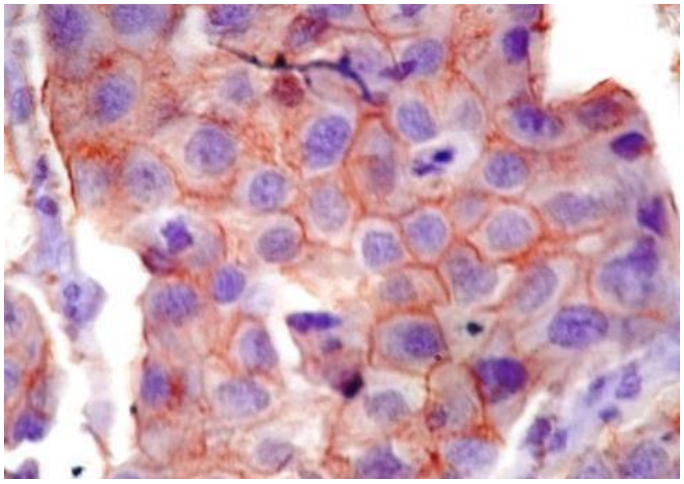


Figure – 25: Weak, moderately complete membranous HER-2/neu immunostaining (score of 2+) in IDC cells (DAB, ×400)

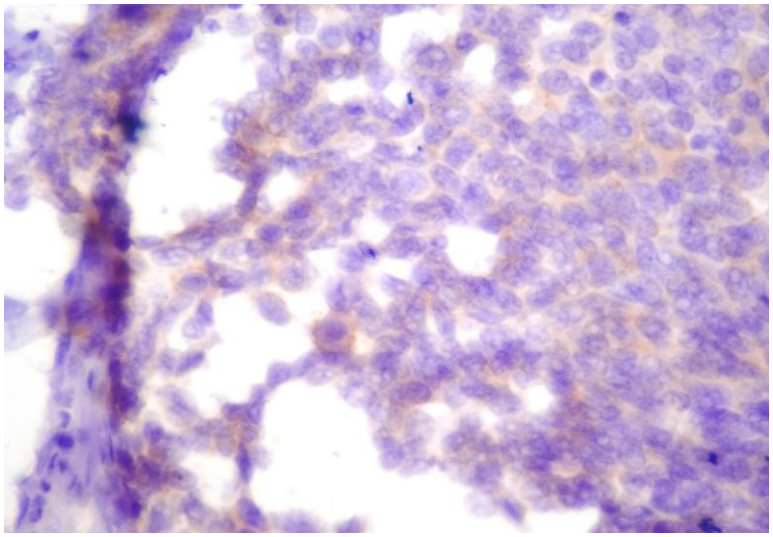


Figure – 26: Faint, incomplete membranous HER-2/neu immunostaining (score of 1+) in IDC cells (DAB, ×400).

Discussion

DISCUSSION

In our study of 50 cases of breast carcinoma, majority of the patients were between the age group of 40- 59 years. This finding is similar to studies of Mansour Al-Moundhri et al[69] (49.6 years), Azizun- Nisa et al[53] (48.3 years), Moradi- Marjaneh M et al[66] (47.4 years), and Lobna Ayadi et al[52] (51.5 years).

In our study carcinoma breast was more common in postmenopausal age group (72%) not similar to studies by Mona M Rashed et al[51] (70%) and Moradi-Marjaneh M et al[66] (58.3%). However in the study by Mansour Al- Moundhri et al[69] only 59.7% of patients were in the postmenopausal age group.

In the present study majority of the cases (64%) had the tumour size ranging from 2- 5 cms, followed by 14 (28%) cases of tumour size of > 5 cms and 4 (8%) cases of < 2 cms. In the study by Azizun- Nisa et al[53] also, 52.7% of tumour were between 2-5 cms and 35.3% were > 5 cms. The study of Mona M Rashed et al[51] also 54% of cases had tumour size of 2-5 cms while 36% had a tumour size of > 5 cms.

Among the 50 cases of invasive carcinoma breast in our study, majority of the cases (49 cases, 98%) were of infiltrating ductal carcinoma, NOS type and 1 case (2%) was invasive papillary carcinoma.

Similarly, in the study by Farid Saleh et al[70] the most common histologic subtype was infiltrating ductal carcinoma, NOS type (98%) and 2% of invasive papillary carcinoma. In the study by Lobna Ayadi et al[52] also, 84% were of infiltrating ductal carcinoma type and 16% were of non- ductal type.

Table – 21 : Table showing comparison of NSBR grade between various studies

NSBR Grade	Mona ashed et al[51] 2007	Saleh F et al[70] 2007	Azizun-Nisa[53] et al 2008	Moradi M et al[66] 2008	Our study 2015
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
1.	05 (10%)	17(10.2%)	10 (6.7%)	28 (10.5)	3 (6 %)
2.	27 (54%)	84(50.6%)	83 (55.3%)	156 (58.6)	45 (90 %)
3.	18 (36%)	65(39.2%)	57 (38%)	82 (30.8)	2 (4 %)

Of the 50 cases of invasive carcinoma breast studied, majority of the cases (45 cases, 90%) were of grade 2, followed by 3 cases (6%) of grade 1. The least number of cases (4%) were of grade 3 (Table -9 , 18, Figure - 17). Similar findings were noted in the studies of Mona Rashed et al[51] (2007), Saleh F et al (2007)[70], Azizun- Nisa et al[53] (2008) and Moradi M et al[66] (2008) were majority in grade 2.

Of the 50 cases of carcinoma breast, majority (24 cases, 48%) were of stage N0, followed by 14 (28%) cases of stage N1, 8 (16%) cases of stage N2 and 4 (8%) cases of stage N3 (Table-10, Figure- 9). In contrast, Mohammad Naeem et al[71] found that majority of the tumours belonged to N3 stage (41.7%) followed by N2 (37.5%), N0 (12.5%) and N1 (8.3%).

Table – 22 : Table showing comparison of NPI between various studies

Nottingham prognostic index (NPI)	Blamey et al[17] 1980 (892 cases)	Blamey et al[17] 1990 (2238	Our study 2015 (50 cases)
EPG	12%	15%	04%
GPG	19%	21%	16%
MPG I	29%	28%	28%
MPG II	24%	22%	32%
PPG	11%	10%	18%
VPG	05%	04%	2%

The NPI in the 50 cases of carcinoma breast showed that most of the patients were in the moderate prognostic group II (32%) . Out of 50 cases, only 2 (4%) and 1 (2%) cases were in excellent and very poor prognostic groups respectively (Table-11, Figure- 10). In comparison, the study by Blamey et al[17] in 1980 and 1990 showed that majority of the cases were in MPG I.

The Her2/neu was expressed in 14 (28%) out of 50 cases of carcinoma breast in the present study. The Her2/neu expression in various studies varied from 15% to 93.4% as shown in table 12.

Table – 23: Table showing comparison of incidence of Her2/neu expression

Study (year)	Incidence of Her2/neu expression
Lovekin C et al[68] (1991)	70/480 (15.0)
Mona Rashed et al[51] (2007)	13/50 (26.0)
Saleh et al[70] (2007)	155/166 (93.4)
Tatjana Ivkovic et al[58] (2007)	23/120 (20.0)
Moradi M et al[66] (2008)	165/319 (51.7)
Azizun- Nisa et al[53] (2008)	56/150 (37.4)
Ayadi et al[52] (2008)	28/155 (18.1)
Vaidyanathan K K et al[48] (2010)	159/368 (43.2)
Ambroise et al[3] (2011)	87/321 (27.1)
Resit Dogan K et al[72] (2011)	12/59 (20.3)
Ahmed et al[55] (2011)	42/137 (30.6)
Huang et al[47] (2012)	241/1362 (17.7)
Our study (2015)	14/50 (28.0)

Maximum Her2/neu positivity was seen in the age group of 40-59 years (78.6%). No statistically significant association of Her2/neu with age was noted in the present study (p value = 0.673).

The results in our study showed that Her2/neu expression increased with age. A study by Al- Moundhri et al[69] (2003) also showed similar results where 12 out of 13 patients with positivity for Her2/neu were > 40 years of age. However many other studies showed increased Her2/neu expression in younger age group (Almasri et al[7] [2005], Farid Saleh et al[70] [2007] and Moses Ambroise et al[3] [2011]). Studies byH J et al[47] (2005), Mona Rashed et al[51] (2007) and Vaidyanathan K K et al[48]

(2010) showed no correlation of Her2/neu expression with age. Patients in post menopausal age group showed more Her2/neu positivity (64.3%) than pre menopausal age group patients (35.7%). No statistically significant association of Her2/neu with menopausal status was noted in the present study (p value = 0.449). Similarly studies by Rashed M et al[51] (2007) and Vaidyanathan K K[48] et al[48] (2010) showed no statistically significant correlation of Her2/neu expression with menopausal status.

Survival analysis done by Vaidyanathan K K et al[48] (2010) revealed that Her2/neu overexpression is poor prognostic indicator. At 40 months of follow up 83.8% of Her2/neu negative individuals had disease free survival whereas only 46.1 % of Her2/neu positive individuals had disease free survival.

Table – 24: Table showing comparison of Her2/neu expression with tumour size

Size	Mona Rashed et	Saleh et al[70]	Tatjana et al[58] 2007	Azizun- Nisa et al[53] 2008	Our study
≤ 2 cms	00.0	28.0	06.3	22.2	14.3
2- 5cms	08.0	84.3	29.5	21.5	50.0
> 5 cms	38.0	92.3	46.2	30.2	35.7
	p = 0.0115	p < 0.005	p = 0.001	p < 0.001	p = 0.374

Tumour size is one of the most useful predictors of behaviour of breast carcinoma. In the present study, while correlating the Her2/neu positivity with the size of the tumour, 2 cases with tumour size < 2 cms were positive for Her2/ neu.

Majority of Her2/neu positivity was seen in with the tumour size between 2- 5 cms (50%), followed by 35.7% Her2/neu positivity was seen with the tumour size > 5 cms (Table 17). However no statistically significant association of Her2/neu with tumour size was noted in the present study (p value = 0.374).

The higher rates of Her2/neu overexpression in larger tumour size have also been documented in some studies as depicted in Table 28 and all of them showed a statistically significant association of Her2/neu with tumour size.

While correlating the over expression of Her2/neu with the histologic subtype, all the 14 positive cases of Her2/neu was seen in infiltrating ductal carcinoma, NOS type. None of the other types showed HER-2 positivity. Similarly, 90.9% and 71.7% of infiltrating duct carcinoma (NOS type) were positive for Her2/neu in the studies done by Naeem et al[71] (2008) and Saleh F et al[70] (2007). In contrast, the study by Ayadi et al[52] (2008) showed 16.8% and 25% Her2/neu positivity in ductal and non ductal carcinomas respectively.

Table – 25: Table showing relationship between histologic grades and Her2/neu positivity

Study	**Grade 1**(%)	**Grade 2**(%)	**Grade 3**(%)	**p value**
Lovekin et al[68] (1991)	03.0	11.0	21.0	<0.0001
Mona Rashed et al[51] (2007)	00.0	04.0	22	0.00001
Saleh et al[70] (2007)	05.9	92.9	87.7	< 0.005
Tatiana et al[58] (2007)	00.0	18.1	47.6	< 0.0001
Moradi M M et al[66] (2007)	46.0	46.6	62.0	0.016
Azizun- Nisa[53] et al (2008)	00.0	22.9	31.6	< 0.001
Naeem M et al[71] (2008)	00.0	18.2	81.8	>0.05
Resit Dogan et al[72] (2011)	00.0	23.1	27.3	0.184
Our study (2015)	00.0	100.0	00.0	0.340

While correlating the over expression of Her2/neu with the histological grading, all the Her2/neu positive tumors were grade 2 tumors. This suggests that the percentage of expression of Her2/neu is more frequent in higher grade breast carcinomas than in lower grade. The studies by various authors shown in the (Table 18) also showed similar results.

No statistically significant association of Her2/neu with histologic grade was noted in the present study (p value = 0.102) similar to the studies by Naeem et al[71] (2008) and Resit Dogan et al[72] (2011). Unlike our study, most of the studies by Lovekin et al[68] (1991), Mona Rashed et al[51] (2007), Saleh et al[70] (2007), Tatjana et al[58] (2007), Moradi M M et al[66] (2007) and Azizun- Nisa et al[53] (2008) showed statistically significant association of Her2/neu with histologic grade.

The correlation of Her2/neu with NPI in the 50 cases of carcinoma breast showed that most of the Her2/neu positive cases were seen in the moderate prognostic group II (50%). None of the cases in excellent group were positive for Her2/neu (Table-19, Figure- 18). No statistically significant association of Her2/neu with NPI was noted in the present study (p value = 0.235).

The Nottingham Prognostic Index (NPI) is a well established and widely used method of predicting survival of operable primary breast cancer. The NPI is compiled from grade, size and lymph node status of the primary tumour. No studies correlating the NPI with Her2/neu status with best of our knowledge has been done so far. Further studies have to be done to determine the significance of correlation of Her2/neu with NPI and to find if this correlation has an advantage over NPI alone in predicting the survival rate of breast carcinoma patients.

Of the 14 Her2/neu positive cases in primary tumour of our study, 12(85.7%) cases showed metastases in the lymph nodes and only 2(14.3%) cases showed no metastases in the lymph nodes suggesting that Her2/neu positivity increases the risk of metastases. Statistically significant association of Her2/neu with lymph node staging was noted in the present study (p value = 0.012).

In the studies by Tatjana et al[58] (2007), Moradi M M et al[66] (2007), Azizun-Nisa et al[53] (2008) and Naeem M et al[71] (2008) also showed increased expression of Her2/neu in cases with lymph node involvement and only study Azizun- Nisa et al[53] (2008) had a statistically significant correlation (Table 26).

Table – 26: Table showing Her2/neu expression in relation to lymph node involvement

Study	Lymph node negative (%)	Lymph node positive (%)	p value
Tatjana et al[58] (2007)	47.8	52.2	>0.05
Moradi M M[66] et al (2007)	23.1	76.9	>0.05
Azizun- Nisa[53] et al (2008)	10.7	89.3	<0.001
Naeem M[71] et al (2008)	09.0	91.0	>0.05
Al- Moundhri et al[69] (2003)	61.5	38.5	0.8
Our study (2015)	14.3	85.7	0.012

Conclusion

CONCLUSION

Her2/neu overexpression participates in the pathogenesis of breast carcinomas. They are indicators of poor prognosis in breast carcinoma and usually correlated with histological grade and stage components of breast carcinomas.

Our study evaluated Her2/neu positive immunoreactivity and correlated with established prognostic factors like clinicopathological parameters, NSBR grade and NPI in 50 cases of carcinoma breast.

According to the data of our study, a significant statistical association was found between Her2/neu and presenting symptoms such as pain and nipple discharge, and lymph node status.

Although no significant statistical association of Her2/neu with tumour size and grade was seen, Her2/neu expression was more frequent in cases with tumour size more than 2 cms and higher grades. This perhaps is attributed to the study of limited number of cases.

No statistical correlation of Her2/neu with menopausal status and NPI group was noted. Her2/neu positive immunoreactivity was more frequently seen in infiltrating duct carcinoma, NOS type.

In conclusion, our study indicates that Her2/neu may be a more powerful predictor for poor prognosis as its expression is associated with important prognostic parameters like increased tumour size, high tumour grade, high NPI score and lymph node involvement.

In future it is necessary to carry out studies with large samples of carcinoma breast and using other molecular prognostic markers to evaluate the prognosis and to provide better therapeutic options.

Summary

SUMMARY

- In the present study, 50 mastectomy specimens of carcinoma breast with axillary clearance were studied from November 2014 to august 2015. The gross and microscopic features with special emphasis on various clinicopathological prognostic parameters were studied.

- All these cases were subjected to IHC for Her2/neu, the results of which were correlated with various clinicopathological prognostic parameters of carcinoma breast.

- Majority of the cases were in the age group 40-59 years (70%).

- Majority of cases had a tumour size of 2 to 5 cms (64%).

- Majority of the cases (98%) were of infiltrating ductal carcinoma NOS type and 2% of invasive papillary carcinoma.

- Majority of cases (90%) were of NSBR grade 2.

- Majority of the cases (52%) showed axillary lymph node metastases.

- Majority of the cases were in the MPG II (32%) group, followed by MPG I (28%), PPG (18%), GPG (16%), EPG (4%) and VPG (2%) groups.

- A statistically significant association of Her2/neu with lymph node status was noted in the present study (p value = 0.012).

- No statistically significant association of Her2/neu with menopausal status was noted in the present study (p value = 0.449).

- No statistically significant association of Her2/neu with tumour size was noted in the present study (p value = 0.374). However the percentage of the Her2/neu expression was more in tumour size of > 2 cms.

- Her2/neu expression was seen only in infiltrating duct carcinoma, NOS type and no statistically significant association of Her2/neu with the histologic subtype was noted in the present study.

- No statistically significant association of Her2/neu with histologic grade was noted in the present study (p value = 0.340). However the percentage of the Her2/neu expression was more in higher grade tumours.

- Statistically significant association of Her2/neu with lymph node staging was noted in the present study (p value = 0.012). However the percentage of the Her2/neu expression was more in cases with lymph node involvement.

- No statistically significant association of Her2/neu with NPI group was noted in the present study (p value = 0.235).

Bibliography

BIBLIOGRAPHY

1. Rosai J. Breast. Rosai and Ackerman,,s Surgical Pathology. 9th ed: Missouri. Elsevier; 2004. 1763-1827.

2. Tavassoli FA, Devilee P. WHO classification of tumours- Pathology and genetics tumours of the breast and female genital organs. Lyon: International agency for research on cancer (IARC press); 2003.

3. Moses Ambroise, Mitra Ghosh, VS Mallikarjuna, Ann Kurian. Immunohistochemical Profile of Breast Cancer Patients at a Tertiary Care Hospital in South India. Asian Pacific J Cancer Prev, 12, 625-629

4. Lester SC. The Breast. In: Kumar V, Abbas AK, Fausto N, Aster JC editors. Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia: Elsevier; 2010, 1073-90.

5. Guidelines for management of breast cancer/by WHO Regional Office for the Eastern Mediterranean: EMRO Technical Publications Series; 31.

6. Kyle Bradley T. Prognostic and predictive markers in breast carcinoma. CAP Cancer Committee 2007.

7. Almasri NM, Hamad MA. Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan. Breast Cancer Research 2005; 7: 598-604.

8. Jambhekar N, Chaturvedi AC, Madur BP. Immunohistochemistry in surgical pathology practice: A current perspective of simple, powerful, yet complex tool. IJPM 2008; 51(1): 2- 12.

9. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL.

Human breast cancer: correlation of relapse and survival with amplification of the Her2/neuoncogene. Science 1987; 235(4785): 177-82.

10. Rani James, Thriveni K, Lakshmi Krishnamoorty, Vijaylaxmi Deshmane. Clinical outcome of adjuvant endocrine treatment according to Her2/neustatus in breast cancer. Indian Journal of Medical Research 2011; 133: 70-5.

11. Antonio C Wolff, Elizabeth M, Hammond H, Jared N Schwartz, Karen L Hagerty, Craig Allred D et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer - College of American pathologists and American society of clinical oncology 2006. Archives of Pathology Laboratory Medicine 2007; 131.

12. Timothy W. Jacobs, Allen M. Gown, Hadi Yaziji, Melissa J. Barnes, Stuart J. Schnitt. Her2/neuProtein Expression in Breast Cancer Evaluated by Immunohistochemistry A Study of Interlaboratory Agreement. Am J Clin Pathol 2000; 113: 251- 258.

13. William LD. History of Breast Cancer, In: David JW editor. Breast Cancer, 2nd ed. USA:Wallsworth publishing company; 2006: 1-15.

14. Parham DM. Mitotic activity and histological grading of breast cancer. Pathol Annu 1995; 30: 189-207.

15. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. Histopathology 1991; 19: 403-10.

16. Emad Rakha A, Maysa El-Sayed E, Andrew Lee HS, Christopher Elston W, Matthew Grainge J, Zsolt Hodi et al. Prognostic significance of nottingham histologic grade in invasive breast carcinoma. Journal of Clinical Oncology 2008; 26(19): 3153-8.

17. Blamey RW, Ellis IO, Pinder SE, Lee AHS, Macmillan RD, Morgan DAL et al. Survival of invasive breast cancer according to the Nottingham Prognostic Index in cases diagnosed in 1990–1999. Eur J Cancer 2007; 43:1548–55.

18. Ellis IO, Pinder SE, Lee AHS, Elston CW. In: Fletcher CDM editor. Diagnostic Histopathology Of Tumors. 2nd ed. New York Churchill Livingstone; 2000. 865-910.

19. Clark GM. Do we really need prognostic factors for breast cancer? Br Cancer Res Treat 1994; 30: 117-26.

20. Page DL. Prognosis and breast cancer. Recognition of lethal and favorable prognostic types. Am J Surg Pathol 1991; 15: 334-49.

21. Jones D, Fletcher C D M. How shall we apply the new biology to diagnostics in surgical pathology? J Pathol 1999; 187: 147-54.

22. Adami HO, Malker B, Holmberg L, Persson I, Stone B. The relation between survival and age at diagnosis in breast cancer. N Engl J Med 1986; 315: 559-63.

23. Robson M. Are BRCA1- and BRCA2- associated breast cancers different? Prognosis of BRCA1- associated breast cancer. J Clin Oncol 2000; 18: 113-8.

24. Carter CL, Allen C, Henson DE. Relation of tumor size, lymph node status and survival in 24,740 breast cancer cases. Cancer 1989; 63: 181-7.

25. McDivitt RW, Boyce W, Gersell G. Tubular carcinoma of the breast. Am I Surg Pathol 1982; 6: 401- 11.

26. Cooper H S, Patchefsky A S, Krall R A. Tubular carcinoma of the breast. Cancer 1978; 42: 2334-42.

27. Carstens PHB, Greenberg RA, Francis D, Lyon H. Tubular carcinoma of the breast. A long term follow up. Histopathology 1985; 9: 271-80.

28. Clayton F. Pure mucinous carcinomas of the breast: morphologic features and prognostic correlates. Hum Pathol 1986; 17: 34-9.

29. Lee BJ, Hauser H, Pack GT. Gelatinous carcinoma of the breast. Surg Gynecol Obstet 1934; 59: 841-50.

30. Page DL, Dixon IM, Anderson TI. Invasive cribriform carcinoma of the breast. Histopathology 1983; 7: 525-36.

31. Bloom HJC, Richardson WW, Fields JR. Host resistance and survival carcinoma of the breast: A study of 104 cases of medullary carcinoma in a series of 1411 cases of breast cancer followed for 20 years. Br Med J 1970; 3: 181-8.

32. Ridolfi RL, Rosen PP, Port A. Medullary carcinoma of the breast - a clinicopathological study with a ten year follow up. Cancer 1977; 40: 1365-85.

33. Haagensen CD, Lane N, Lattis R, Bodian C Lobular neoplasia (so called lobular carcinoma in situ) of the breast. Cancer 1978: 42: 737-69.

34. Fisher ER, Gregaria R M, Redmond C, Fisher B. Tubulolobular invasive breast cancer: A variant of lobular invasive cancer. Hum Pathol 1977; 8: 679-83.

35. Page DL, Ellis IO. Histologic grading of breast cancer. Am J Clin Pathol 1995; 2: 123-4.

36. Fitzgibbons PL, Page DL, Weaner D, Thor AD, Allred C, Clark GM, et al. Prognostic factors in breast cancer. College of American Pathologists consensus statement 1999. Arch Pathol Lab Med 2000; 124: 966-78.

37. Doussal VL, Tubiana-Hulin M, Friedman S, Hacene K, Spyratos F, Brunet M. Prognostic value of histologic grade nuclear components of Scarff-

Bloom- Richardson (SBR). An improved score modification based on a multivariate analysis of 1262 invasive ductal breast carcinoma. Cancer 1989; 64: 1914-21.

38. Frierson HF, Wolber RA, Berean KW, Franquemont DW, Gaffey MJ, Boyd JC, et al. Am J Clin Pathol 1995; 103: 195-8.

39. Carriaga M, Henson DE. The histologic grading of cancer. Cancer 1995; 75: 406-21.

40. Meena SP, Hemarajani DK, Joshi N. A comparative and evaluative study of cytological and histological grading system profile in malignant neoplasm of Breast: an important prognostic factor. Indian J Pathol Microbiol 2005; 49: 199-202.

41. Anderson E, Clarke RB, Howell A. Estrogen responsiveness and control of normal human breast proliferation. J Mammary Gland Biol Neoplasia 1998; 3: 23-35.

42. Taylor CR, Shi SR, Barr NJ. In: Dabbs D editor. Diagnostic immunohistochemistry.3rded. Philadelphia. Elsevier 2010; 1-2.

43. Laura C. Collins, Stuart J. Schnitt Breast Editors: Mills, Stacey E. Title: Histology for Pathologists, 3rd Edition .Copyright Â©2007 Lippincott Williams & Wilkins.

44. Francis E, Sharkey D, Allred C, Valente PT. Breast. In: Damjanov I, Linder J. editors Anderson,,s pathology, 10th ed. Missouri; Elsevier: 1996: 2377.

45. Kent Osborne C, Richard M Elledge, Suzanne AW Fuqua. Estrogen receptors in breast cancer therapy. Science and Medicine; 1996.

46. Allen Gown M. Current issues in ER and HER2 testing by IHC in breast cancer. Modern Pathology 2008; 21: S8–15.

47. H J Huang, P Neven, M Drijkoningen, R Paridaens, H Wildiers, E Van

Limbergen, P Berteloot, F Amant, I Vergote, M R Christiaens. Association between tumour characteristics and Her2/neu by immunohistochemistry in 1362 women with primary operable breast cancer . J Clin Pathol 2005; 58: 611–616.

48. Vaidyanathan K, Kumar P , Reddy CO , Deshmane V , Somasundaram K, Mukherjee G. ErbB-2 expression and its association with other biological parameters of breast cancer among Indian women. Indian Journal of Cancer 2010; 47 (1): 8-15.

49. Schubert D, Heinemann S, Carlisle W, Tarikas H, Kimes B, Patrick J. Clonal cell lines from the rat central nervous system. Nature 1974; 249: 224-27.

50. Van de Vijver M, Van de Bersselaar R, Devilee P, Cornelisse C, Peterse J, Nusse R. Amplification of the neu (c-erbB-2) oncogene in human mammmary tumors is relatively frequent and is often accompanied by amplification of the linked c-erbA oncogene. Molecular Cell Biology 1987; 7(5): 2019-23.

51. Mona M Rashed, Noha M Ragab, Manal K Galal. The Association Of Her2/neuOver-Expression In Relation To P53 Nuclear Accumulation, Hormonal Recceptor Status And Common Clinico-Pathological Prognostic Parameters In A Series Of Egyptian Women With Invasive Ductal Carcinoma. Eur J Gen Med 2007; 4(2):73-79.

52. Lobna Ayadi, Abdelmajid Khabir, Habib Amouri, Sondes Karray, Abdallah Dammak, Mohamed Guermazi et al. Correlation of HER-2 over-expression with clinico-pathological parameters in Tunisian breast carcinoma. World Journal of Surgical Oncology 2008; 6: 112.

53. Nisa A, Bhurgri Y, Raza F, Kayani N. Comparison of ER, PR and

Her/2 /neu reactivity pattern with histologic grade, tumor size and lymph node status in breast cancer. Asian J Cancer prevention 2008; 9: 553-6.

54. Mudduwa LKB. Quick score of hormone receptor status of breast carcinoma: correlation with other clinicopathological parameters. IJPM 2009; 52(2): 159- 163.

55. Hussain Gadelkarim Ahmed, Mohammed Ali Al-Adhraei and Abdullah Kasim Al-Thobhani. Correlations of Hormone Receptors (ER and PR), Her2/neu and p53 Expression in Breast Ductal Carcinoma among Yemeni Women. The Open Cancer Immunology Journal 2011; 4: 1-9.

56. Valérie Pawlowski, Françoise Révillion, Mohamed Hebbar, Louis Hornez, Jean- Philippe Peyrat. Prognostic value of the type I growth factor receptors in a large series of human primary breast cancers quantified with a real-time reverse transcription-polymerase chain reaction assay: Laboratoire d‟Oncologie Moléculaire Humaine, Centre Oscar Lambret, 59020 Lille Cédex, France.

57. Shinichi Tsutsui, Shinji Ohno, Shigeru Murakami, Yoichi Hachitanda, Shinya Oda. Prognostic value of c-erbB2 expression in breast cancer. Journal of Surgical Oncology 2002; 79(4): 216–23.

58. Tatjana Ivkovic. Correlation of Her2/neuprotein overexpression with other prognostic and predictive factors in invasive ductal breast cancer. Anticancer Research 2007; 21: 673-8.

59. John Alfred Carr, Suzzane Havstad, Richard Zarbo. The association of Her2/neuamplification with breast cancer recurrence. Archives of Surgery 2000; 135: 1469-74.

60. Amanda McCann H, Peter Dervan A, Myra O'Regan, Mary Codd B, William Gullick J, Brendan Tobin MJ et al. Prognostic significance of c-erbB-2 and estrogen receptor status in human breast cancer. Cancer 2000; 88(4): 804–13.

61. Heikki Joensuu, Jorma Isola, Mikael Lundin, Tiina Salminen, Kaija Holli, Vesa Kataja et al. Amplification of erbB2 and erbB2 expression are superior to estrogen receptor status as risk factors for distant recurrence in pT1N0M0 breast cancer-A nationwide population-based Study. Clinical Cancer Research 2003; 9: 923-30.

62. Parvinee Suwanagool. Emerging technologies for HER2 testing. Siriraj Medical Journal 2009; 69(3): 172-74.

63. Selvarajan S, Bay BH, Chng MJ, Tan PH. The hercep test and routine C-erbB2 immunohistochemistry in breast cancer: Any difference. Annals of Academy of Medicine Singapore 2004; 33: 473-76.

64. Benöhr P, Henkel V, Speer R , Vogel U, Sotlar K, Neubauer H et al. Her2/neuexpression in breast cancer – A comparison of different diagnostic methods. Anticancer research 2005; 25: 1895-1900.

65. Love RR, Duc NB, Havighurst TC et al. Her2/neu overexpression and response to oophorectomy plus tamoxifen adjuvant therapy in estrogen receptor-positive premenopausal women with operable breast cancer. Journal of Clinical Oncology 2003; 21: 453-7.

66. Moradi-Marjaneh M, Homaei-Shandiz F, Shamsian SAA, Eftekhar-Zadeh Mashhadi I, Hedayati-Moghadam MR. Correlation of HER2/neu Over Expression, p53 Protein Accumulation and Steroid Receptor Status with Tumor Characteristics: An Iranian Study of Breast Cancer Patients. Iranian J Publ Health 2008; 37(3): 19-

28.

67. V. Ludovini, S. Gori, M. Colozza, L. Pistola, E. Rulli, I. Floriani et al. Evaluation of serum HER2 extracellular domain in early breast cancer patients: correlation with clinicopathological parameters and survival. Annals of Oncology 2008; 19: 883–890.

68. Lovekin C, Ellis IO, Locker A, Robertson JFR, Bell J, Nicholson R et al. c- erbB-2 oncoprotein expression in primary and advanced breast cancer. Br. J. Cancer 1991; 63: 439-443.

69. Mansour Al-Moundhri, V Nirmala, K Al-Mawaly, S Ganguly, I Burney, A Rizvi et al. Significance of p53, Bcl-2 and Her2/neuProtein Expression in Omani Arab Females with Breast Cancer. Pathology oncology research 2003; 9(4). Article is available online at http://www.webio.hu/por/2003/9/4/0226

70. Saleh F, Abdeen S. Pathobiological features of breast tumours in the State of Kuwait: a comprehensive analysis. Journal of Carcinogenesis 2007; 6:12. Article is available online at http://www.carcinogenesis.com/content/6/1/12

71. Naeem M, Nasir A, Aman Z, Ahmad T, Samad T. frequency of Her2/neureceptor positivity and its association with other features of breast cancer. J Ayub Med Coll Abbottabad 2008;20(3).

72. Reşit Doğan K, Ahmet M, Unal E, Julide Saliha C, Pervin D, Yavuz Hakan O. An analysis of Her2/neugene status in invasive ductal carcinomas using immunohistochemistry and fluorescence in situ hybridization. Turk J Med Sci 2011; 41 (5): 809-819.

Annexure

PROFORMA

Name :

Age/Sex:

IP no:

Address:

Contact no:

Date of Admission:

Date of Discharge:

Presenting complaints:

h/o. mass

h/o. pain

h/o. discharge from the nipple

Past history:

h/o. Diabetes/Hypertension/Liver/Kidney/Thyroid diseases

h/o. Radiation exposure

h/o.Previous surgeries

Diet history: Vegetarian/Non vegetarian

Family history:

Menstrual history:

Age at menarche

Irregular mensus – any treatment taken specify

Hormone replacement: yes/no

Estrogen only/estrogen + progesterone

OCP intake: yes/no

Age at birth of 1st child

Breast feeding

No. Of children

Age at menopause

General Examination:

Anaemia

Obesity

Blood Pressure

Pulse rate

Cardiovascular system

Respiratory system

Examination of abdomen

Examination of breast:

Investigations:

Basic blood investigations

USG abdomen

USG breast

FNAC

Trucut biopsy

Metastatic workup

Clinical staging (AJCC):

Pathological factors:

Gross features:

Location

Multicentricity

Bilaterality

Tumor size

Tumor margins

Surgical margins

Underlying muscle involvement

Skin involvement

Nipple involvement

Lymph node

Microscopy

Insitu/Invasive

Lymphovascular invasion

Lymph node

Surgical margins

Underlying muscle involvement

Skin involvement

Nipple involvement

Histological subtype

Necrosis

Fibrosis

Stromal reaction

Pagetoid spread

Histological grading:

Tubules

Nuclear pleomorphism

Mitosis

Total score

NSBR grade: I/II/III

HER2/neu

NPI

Type of therapy:

Surgery

Chemotherapy

Radiotherapy

சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு :

A STUDY OF EXPRESSION OF HER2/neu IN CARCINOMA BREAST WITH
ஆராய்ச்சி REFERENCE TO CLINICOPATHOLOGICAL FEATURES AND PROGNOSTIC INDEX

கழபாகக்ம மருததுவக கலலூரி
சென்னை – 600 010.

பங்கு பெறுபவரின் பெயர் : வயது :

பங்கு பெறுபவரின் எண். :

பங்கு பெறுபவரது இதனை (✓) குறிக்கவும்

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களைப் பெறவும் வாய்ப்பளிக்கப்பட்டது. ☐

நான் இவ்வாய்வின் தன்னிச்சையாகத்தான் பங்கேற்கிறேன். எந்தக் காரணத்தினாலோ எந்தக் கட்டத்திலும் எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகிக் கொள்ளலாம் என்று அறிந்து கொண்டேன். ☐

இந்த ஆய்வு சம்மந்தமாகவோ, இதைச் சார்ந்த மேலும் ஆய்வு மேற்கொள்ளும்போது இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளைப் பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன். ☐

இந்த ஆய்வின் மூலம் கிடைக்கும் தகவல்களையும், பரிசோதனை முடிவுகளையும் மற்றும் சிகிச்சை தொடர்பான முடிவுகளையும் மருத்துவர் மேற்கொள்ளும் ஆய்வில் பயன்படுத்திக் கொள்ளவும் அதைப் பிரசுரிக்கவும் என் முழு மனதுடன் சம்மதிக்கிறேன். ☐

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக்கொள்கிறேன். எனக்குக் கூறப்பட்ட அறிவுரைகளின்படி நடந்து கொள்வதுடன், இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்றும் உறுதியளிக்கிறேன். என் உடல் நலம் பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத நோய்க்குறி தென்பட்டாலோ உடனே அதை மருத்துவ அணியிடம் தெரிவிப்பேன் என உறுதி அளிக்கிறேன். ☐

பங்கேற்பவரின் கையொப்பம் .....இடம் ..... தேதி
கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம் ..... தேதி

ஆய்வாளரின் பெயர் .....

KEY TO MASTER CHART

Sl No	–	serial number
IP no	–	In patient number
Nip Dis	–	Nipple discharge
Menopaus	–	Menopause
Clin Stage	–	Clinical stage
Tum size	–	Tumour size
Tubule	–	Tubule formation
Nuc pleo	–	Nuclear pleomorphism
LN	–	Lymph node
NPI	–	Nottingham Prognostic Index.

MASTER CHART

Sl. No	NAME	IP NO	AGE	LUMP	PAIN	NIP DIS	MNP	TUM SIZE	HIS TYPE	TUBULES	NUC PLEO	MITOSIS	GRADE	TOTAL LN	LN METS	NPI SC	NPI GR	HER2/neu
1	FARDUNISHA	37407	48	1	0	0	0	5	IDC	3	2	2	2	23	0	4	MPG I	0
2	AYESHA	25716	42	1	1	0	0	3.5	IDC	2	2	2	2	27	21	5.7	PPG	0
3	ANSARI	13596	55	1	0	0	1	5	IDC	2	2	2	2	14	2	5	MPG II	3+
4	GAJENDRI	28235	55	1	0	0	1	5.3	IDC	3	2	2	2	14	7	6.06	PPG	0
5	INDRA	14685	53	1	1	1	1	1.5	IDC	2	2	2	2	5	1	3.3	GPG	3+
6	MALLIGA	34847	55	1	0	0	1	7	IDC	2	3	2	2	4	3	5.4	MPG II	0
7	MANJULA	23011	52	1	0	0	1	2.5	IDC	2	2	2	2	18	0	3.5	MPG I	0
8	POWNU	24098	50	1	0	0	1	4	IDC	2	2	2	2	21	0	3.8	MPG I	1+
9	DEVI	14663	38	1	1	0	0	2.5	IDC	3	2	2	2	11	0	3.5	MPG I	0
10	AMEENA	14022	45	1	1	0	0	2	IDC	2	2	2	2	10	0	3.4	GPG	0
11	CHANDRA	4845	48	1	0	0	1	6	IDC	3	3	2	3	4	0	5.2	MPG II	0
12	VELAMMAL	28236	72	1	0	0	1	2	IDC	2	2	2	2	29	0	3.4	GPG	0
13	PARVATHY	13597	54	1	0	0	1	4	IDC	2	2	2	2	21	5	5.8	PPG	0
14	THARABHAI	23207	60	1	0	0	1	3	IDC	2	2	2	2	17	2	4.6	MPG II	1+
15	MUNIRUNISHA	44376	45	1	0	0	0	2	IDC	2	2	2	2	12	0	3.4	GPG	0
16	SAVITHRI	18484	40	1	0	0	0	4	IDC	2	2	2	2	17	2	4.8	MPG II	3+
17	RANI	30171	41	1	1	0	0	1.5	IDC	2	2	2	2	22	0	3.3	GPG	0
18	SHAKUNTHALA	14686	45	1	0	0	0	4.8	IDC	2	2	2	2	10	5	5.96	PPG	3+
19	VIJAYA	9193	58	1	0	0	1	4.5	IDC	2	2	2	2	21	8	5.9	PPG	0
20	VIRUTHAMBAL	14023	53	1	0	0	1	5	IDC	2	3	2	2	12	3	5	MPG II	3+
21	ANJALAI	36991	47	1	0	0	1	4	IDC	2	2	2	2	38	4	4.8	MPG II	2+
22	NAGAPUSHPAM	41005	48	1	0	1	1	5	IPC	3	2	2	2	15	0	4	MPG I	0
23	MALLIKA	40389	48	1	0	0	1	8	IDC	2	3	2	2	24	8	6.6	VPG	3+
24	ANUSIYA	40487	60	1	0	0	1	4	IDC	2	2	2	2	11	10	5.8	PPG	0
25	REVATHY	45572	50	1	0	0	1	5	IDC	3	2	2	2	23	0	4	MPG I	0

26	PADMINI	29808	46	1	0	0	1	3.5	IDC	2	2	2	2	27	21	5.7	PPG	0
27	RUKMANI	28231	45	1	0	1	0	5	IDC	2	2	2	2	14	3	5	MPG II	3+
28	MAHALAKSHMI	23011	56	1	0	0	1	5.3	IDC	3	2	2	2	14	7	5.06	MPG II	0
29	PREMA	23176	47	1	0	0	1	7	IDC	2	3	2	2	18	0	4.4	MPG I	0
30	BAKKIYAM	21599	51	1	0	0	1	2.5	IDC	3	2	2	2	11	0	3.5	MPG I	0
31	VALLIKANNU	20062	30	1	0	0	1	2.7	IDC	2	2	2	2	21	0	3.54	MPG I	0
32	RUBI	13121	38	1	0	0	0	2	IDC	2	2	2	1	10	0	2.4	EPG	0
33	SATHIYA	9871	50	1	0	0	1	6.2	IDC	3	3	2	3	4	0	5.24	MPG II	0
34	PARAMESWARI	5721	51	1	1	1	1	1.7	IDC	2	2	2	2	5	2	3.34	GPG	3+
35	RABIYA BEE	5780	72	1	0	0	1	4.7	IDC	2	2	2	2	10	0	3.94	MPG I	3+
36	SHANTHI	37408	55	1	1	0	1	4	IDC	2	2	2	2	11	10	5.8	PPG	3+
37	DHANALAKSHMI	36992	47	1	0	0	1	2	IDC	2	2	2	2	17	2	4.4	MPG II	0
38	ANJALA	41006	55	1	0	0	1	4	IDC	2	2	2	2	21	5	5.8	PPG	0
39	KARPAGAVALLI	40388	59	1	0	0	1	3	IDC	2	2	2	2	17	2	4.6	MPG II	0
40	THALABAI	40486	63	1	0	0	1	2	IDC	2	2	2	2	12	0	3.4	GPG	0
41	LAKSHMI	45573	56	1	0	0	1	4	IDC	2	2	2	2	17	2	4.8	MPG II	0
42	UMA	29809	48	1	0	0	1	1.5	IDC	2	2	2	1	5	0	2.3	EPG	0
43	USHA DEVI	282232	33	1	1	1	0	5	IDC	2	2	2	2	11	2	5	MPG II	3+
44	TAMIL SELVI	23012	37	1	1	0	0	3	IDC	2	2	2	2	5	2	4.6	MPG II	3+
45	ABITHA BEE	23177	48	1	0	0	1	4	IDC	2	2	2	2	4	0	3.8	MPG I	0
46	MANABOOBJAN	21598	37	1	0	0	0	4.2	IDC	2	2	2	2	14	3	4.84	MPG II	1+
47	KAMALA	20063	66	1	0	0	1	3.2	IDC	2	2	2	2	23	0	3.64	MPG I	0
48	PUSHPA	13122	65	1	0	0	1	3	IDC	2	2	2	2	11	0	3.6	MPG I	1+
49	ALLI	9872	42	1	0	0	0	3	IDC	2	2	2	1	7	0	2.6	GPG	0
50	MEGALA	5722	48	1	0	0	1	3.8	IDC	2	2	2	2	10	0	3.76	MPG I	2+